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STUDIES ON PROPAGATION OF MENGOVIRUS IN L-CELL MONOLAYER CULTURES

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Studies on Propagation of Mengovirus in L-Cell Monolayer Cultures" submitted by Shubdarshan K. Jawanda in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Studies were done on the propagation of Mengovirus strain "M" with particular emphasis on some of the factors which might influence the propagation of the virus in L-cell monolayer cultures.

During the propagation of Mengovirus in L-cell monolayer cultures, virus titres were measured by the plaque assay method. It was observed that the plaques formed by Mengovirus were 2-3 mm in diameter. The maximum attachment of virus to cells occurred within one hour after infection and the yield of the virus continued to increase during the period nine hours to twenty-seven hours after attachment. The maximum virus titre was 1.6×10^9 plaque forming units per milliliter. These preliminary observations did not differ significantly from pre-existing reports on the propagation of Mengovirus

In the course of investigations of factors controlling the propagation of Mengovirus in L-cell cultures, attempts were made to prepare Mengovirus interferon (IF) and to observe its effect on the growth of homologous virus. Preparations which could be assumed to contain Mengovirus IF did not show any effect on the plaque forming ability, virus yield or efficiency of attachment of Mengovirus in L-cell cultures. Partially purified interferon was prepared and assayed to determine whether or not it could cause interference with the growth of Mengovirus. Extracellular and intracellular interferon did not show any effect. Similar preparations of Mengovirus interferon were not effective in suppressing the growth of vaccinia virus in L-cell cultures.

Interference studies between heat-inactivated (at $37^{\circ}C$) Mengovirus, ultraviolet light-inactivated Mengovirus and live Mengovirus were conducted.

No interference was observed between inactivated virus and live virus. However, studies between live Newcastle disease virus (NDV) and live Mengovirus showed complete inhibition of Mengovirus in L-cell cultures. In addition, preparations of Newcastle disease virus interferon showed about 70% reduction in the number of plaques of Mengovirus. It is concluded that Mengovirus does not stimulate the production of detectable amounts of interferon under any of the foregoing experimental conditions. However, Mengovirus is sensitive to the effects of interferon.

Studies were done on the effect of rabbit anti-Mengovirus serum (RAMS), rabbit normal serum (RS) and their respective γ-globulins on Mengovirus, infectious Mengovirus ribonucleic acid and tritium labelled purified Mengovirus infectious ribonucleic acid. These studies revealed that Mengovirus was neutralized by RAMS and immune γ-globulin, whereas infectious Mengovirus ribonucleic acid and purified tritium-labelled infectious ribonucleic acid were rendered non-infective by RAMS, RS, immune γ-globulin and normal γ-globulin. Subsequent studies demonstrated that these latter results were due to serum ribonuclease which was present in each case. Under conditions of cell culture the activity of serum nuclease must, therefore, prevent the survival of extracellular, infectious ribonucleic acid.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CAM	- Chorio-allantoic membrane
CPE	- Cytopathic effect
DNA	- Deoxyribonucleic acid
EBSS	- Earl's balanced salt solution
γ-globulin	- Gamma globulin
HA	- Hemagglutinin
IRNA	- Infectious ribonucleic acid
IF	- Interferon
"IF"	- "Interferon" - used in this thesis to designate hypothetical interferon.
m-RNA	- Messenger ribonucleic acid
ANS reagent	- Molybdic amino-naphthol sulfonic acid reagent
NDV	- Newcastle disease virus
pfu	- Plaque forming units
RAMS	- Rabbit anti-Mengovirus serum
RS	- Rabbit normal serum
RNase	- Ribonuclease
RNA	- Ribonucleic acid

INTRODUCTION

Introduction

Mengovirus

Mengovirus is highly infectious for rodents. Strains of Mengovirus have been obtained from mosquitoes. Mengovirus also was found to be infectious in laboratory work by Dick et al. (1948). They did quite a detailed study on this virus. The infection can be transferred from rodents to man. This virus is closely related to Columbia SK, MM and encephalomyocarditis (EMC) viruses. Ellem and Colter (1961) separated three strains of Mengovirus depending upon the morphology and hemagglutinating characteristics. The strains were "S" (small size plaque producing), "M" (medium size plaque producing) and "L" (large size plaque producing). Strains M and S showed hemagglutinating activity, whereas strain L did not.

Properties

It is an RNA containing virus. It is very small in size, the exact size is not known but thought to be under 30 mμ. Nothing is known about the shape of the virus. It is remarkably heat stable. Even after heating at 80°C for 30 minutes the infectivity is not lost. Virus infectivity can be destroyed by UV light (Rhodes and Van Rooyen, 1962). It can be stored for long periods at -60°C or -70°C without loss of infectivity. The virus grows and produces cytopathic effects in L-cells, Hela-cells, KB-cells and monkey kidney cultures. Histologically, infected animals show degeneration of neurons and myocarditis.

The biochemistry of replication of Mengovirus in L-cells

Mengovirus multiplies rapidly in L-cells, giving a high yield of infectious virus particles per cell. Franklin (1962) found that it could produce maximum titers of 12,000 particles per cell.

Mengovirus, as do other animal viruses, attaches to the cell and then it penetrates into the cell. Different attachment times have been given by different workers. A detailed study on attachment of Mengovirus to L-cells has been done by Colter et al. (1964). They showed that maximum attachment of M and S strains to L-cell monolayer cultures takes place between 10 to 15 minutes, but plaques produced by L Mengo increase progressively from zero to 60 minutes. It is said that Mengovirus synthesis takes place in the cytoplasm of the cell (Franklin and Rosener, 1962).

Viral RNA on entering into the cells acts as a genome and becomes stabilised through the formation of polyribosomes (Tobey, 1964) upon which viral proteins are synthesised. During the action as m-RNA, the virus genome is not degraded nor does it enter into any double-stranded replicative intermediates, but rather may employ a direct base for base copying mechanism (Tobey, 1964). Parental RNA does not incorporate itself into the progeny. A new enzyme, ribonucleic acid polymerase, (Baltimore and Franklin, 1963) is found in the cells after infection with Mengovirus. This enzyme catalyses incorporation of the ribonucleoside monophosphates of adenine, guanine, uracil and cytosine into an acid insoluble form. Mengovirus infection in L-cells causes a rapid decline in the rate of synthesis of both cellular RNA and protein (Baltimore, Franklin and Callender, 1963). Baltimore and Franklin (1962) found that Mengovirus suppresses the activity of DNA-dependent RNA polymerase, which could account for the effects on the synthesis of cell RNA and cell protein.

RNA and viral protein accumulate in the perinuclear region of the

cytoplasm during the early stages of infection. Later inclusions containing RNA and viral protein are observed (Franklin and Rosener, 1962).

Different times are given by different workers for the synthesis and maturation of virus. Brownstein and Graham (1961) have given that maturation of virus begins at 3.5 hours after infection. Release of virus begins at 4 hours and is complete at 7 hours. Homma and Graham (1963) have found that formation of infectious RNA is begun at about 4 hours and is completed at 7 hours. Viral maturation commences at about 6.5 hours and is completed by 9 hours.

L-Cells

Strain "L" was initiated by Earle et al. (1940) from an explant of connective tissue derived from a single 100 day old male mouse of C_3H strain. In the course of serial transfers in plasma cultures, the cell population was treated with methyl-cholanthrene. It eventually produced sarcomas when injected into the subline of origin, the C_3H mouse. Strain L was established on glass surface from the carcinogen treated cultures. A population derived from this strain was called strain L-clone 929. This strain has become widely distributed and was used in the virus propagation studies which are reported in this thesis.

Viral Interference

There is a phenomenon known as viral interference; it is the interference caused by one virus to the growth of another virus. Schlesinger (1959) defined it as such, that at the qualitative level, interference expresses itself as protection against injury, disease, or

death, or in general against manifestations of virulence. It is postulated that interference results uniquely and directly from an association of the interfering virus with susceptible cells and this association is primarily responsible for the inability of the super-infecting virus to multiply and to cause cell injury. More recently it has been found (Isaacs and Lindenmann, 1957) that interference can be caused by a protein, liberated by infected cells, which transfers interference to non-infected cells. This substance was named Interferon (IF) by its discoverers. Viral interference is not completely understood as yet. It is, therefore, very hard to establish any suitable definition of interference at present.

Viral interference was first recognised by McKinney (1929) in plant viruses. He reported that if plants are infected with yellow mosaic virus prior to infection with yellow mosaic tobacco virus, the later would not multiply in the same plants. However, it is interesting to note that Jenner (1804) was probably the first one to observe this phenomenon in human beings. He found that the herpetic infection prevented the vaccinia virus from producing its characteristic lesion.

A similar phenomenon in relation to animal viruses was described (Hoskins, 1935) between neurotropic strain of yellow fever virus and viscerotropic strain of yellow fever virus in Macacus Rhesus monkeys. They inoculated the neurotropic strain of yellow fever and viscerotropic strain of yellow fever both simultaneously and at intervals of 48 hours. In the latter cases, the viscerotropic strain was always inoculated first. They performed seventeen experiments using 48 animals. From these experiments it was found that neurotropic virus appears to have a protective effect on

monkeys when inoculated simultaneously with the viscerotropic strain. Thirteen out of fifteen animals receiving both the viruses simultaneously, survived. Of those two deaths, one was due to viscerotropic yellow fever virus and the other due to some unknown factor. Complete protection was obtained when the time interval was no greater than 20 hours between the two injections. When the neurotropic virus was administered after an interval of 48 hours, there appeared to be no protective effect. The investigators at that time could not give any explanation for the protective effect of the neurotropic virus.

Findlay and MacCallum (1937) confirmed the findings of Hoskins and attempted to explain the findings. They performed nine experiments in all. The first of those experiments confirmed that the neurotropic strain protected against the pantropic strain of yellow fever virus. They injected the monkeys with pantropic strain of yellow fever virus at intervals varying from 10 minutes to 4 hours. Later on these monkeys were inoculated with the neurotropic strain of yellow fever virus. Of these fifteen monkeys, only two died. These findings were compatible with the findings of Hoskin.

They also found that neurotropic strain protected against the viscerotropic strain and postulated that the reverse might not hold true because the inoculation of monkeys with a mixture of neurotropic and pantropic yellow fever viruses resulted in death with encephalitis. Inoculation of hedge-hogs with a mixture of neurotropic and pantropic viruses was followed by the death of hedge-hogs from encephalitis.

Findlay and MacCallum (1937) also tried to determine whether in man there was any interference between viscerotropic and neurotropic

strains. In man also, the experimental evidence suggested that the neurotropic strain protected against the viscerotropic strain. They also found that the interference of the neurotropic strain with the pathogenic effects of the pantropic strain in the Rhesus monkey was not due to an early development of immune bodies either in the serum or in the lymph nodes. They offered the explanation that perhaps the protective effect was due to blocking of certain cells by the first virus and which interfered with the activity of second virus. To test this hypothesis, they inoculated the Rhesus monkeys with a mixture of pantropic yellow fever virus and pantropic Rift Valley fever virus. This resulted in the protection of 7 out of 11 experimental animals.

On the basis of such limited evidence, they offered one possible explanation of interference as shown by the neurotropic strain of yellow fever virus. They postulated that when the cells are already occupied by multiplying virus particles they cannot be invaded by certain other virus particles.

Later on, similar observations were made by many others using various serologically unrelated viruses. Andrews (1942) simplified the experimental system for the studies of interference by propagating viruses in the allantoic cavity of fertile hen's egg or in tissue culture. He showed that WS strain of influenza virus inhibited the growth of a neurotropic variant of the same strain in chick embryo tissue culture.

Another important aspect of the phenomenon was established when it was found that instead of live influenza virus inactivated virus could cause interference. Inactivation could be accomplished by various methods, namely ultraviolet (UV) irradiation (Henle and Henle, 1943),

formaldehyde or heat (Henle and Henle, 1943; Isaacs and Edney, 1950). Inactivated influenza virus (Henle and Henle, 1943; Zeigler, Lavin and Horsfall, 1944) interfered with the growth of other influenza viruses in the allantoic cavity. Inactivation of influenza virus was done under carefully controlled conditions. Heating to 56°C or formaldehyde treatment destroyed most of the infective capacity of the virus, while retaining the interfering activity.

Lennet and Koprowski (1946) observed interference in tissue cultures using minced 8-10 day old chick embryos without central nervous system. They found that 17DD high strain of yellow fever virus was able to inhibit the Asibi strain of yellow fever virus and West Nile virus, even when the latter viruses were added to tissue culture in large amounts. 17DD high strain of yellow fever virus and West Nile virus produced either partial or complete suppression of the growth of the Venezuelan-equine-encephalitis virus, depending upon the dose of the latter. However, the protective effect could be abolished with high dosage of Venezuelan-equine-encephalitis virus. The 17DD high strain of yellow fever virus was able to suppress completely the growth of influenza A virus. Cultures infected with 17DD high strain of yellow fever virus, were examined for the presence of neutralizing antibodies and non-specific antiviral substance. Neither was found to be present. They also observed that virus-free fluid from minced chick embryo tissue cultures infected with 17DD high strain of yellow fever virus imparted resistance to the growth of the above described viruses in fresh tissue cultures.

Isaacs and Edney (1950) studied interference between inactive and active viruses. They used three strains of influenza A virus:

WSE, PR8 and MEL. Influenza B Lee strain, mumps American strain and Newcastle disease virus (NDV) Victorian strain were employed. They used two methods for the inactivation of the virus, formalinization and heating for one hour at 56°C. Experiments were performed with 11-12 day old chick embryos, which were inoculated suballantiocally with inactive virus. Following incubation for one hour at 37°C, they were infected with challenge virus. The eggs were again incubated at 35°C for 42 hours, after which the allantoic fluids were harvested and titrated individually for their hemagglutinin content. After doing many experiments they came to the following conclusions:

1. A high concentration of interfering virus caused more interference than did lower concentrations.
2. A large dose of challenge virus overcame the effect of an interfering virus.
3. Heated MEL virus was an effective interfering agent.
4. Interference was increased by increasing the time interval between times of inoculation of interfering virus and the challenge virus.
5. Both Newcastle disease virus (NDV) and mumps viruses were inhibited by the minimal interfering dose of heat-inactivated influenza virus B, Lee strain.

Tyrrell and Tamm (1955) demonstrated interference between homologous and heterologous strains of influenza A and B. The viruses were grown in chorio-allantoic membrane in vitro. Inactivated influenza A or influenza B virus was prepared for interference experiments by heating at low temperatures (22°C or 32°C). This treatment preserved more interfering activity of the virus than did heating at 56°C, although infectivity

was less effectively eliminated at lower temperatures.

Between 1955 and 1957 many observations of heterologous and homologous viral interference were recorded. Isaacs and Lindenmann (1957) working on interference between different strains of influenza viruses discovered that interference could be produced by a non-viral substance. This substance was found in the allantoic fluids of infected chorio-allantoic membranes (CAM). They named the substance Interferon (IF). It is essential to describe Isaacs and Lindenmann's experiments and results briefly. They used the Melbourne strain of influenza virus A as the interfering virus. It was prepared by heating the virus at 56°C for one hour. This treatment destroyed the infectivity of the virus and retained its interfering activity. Pieces of chorio-allantoic membrane (CAM) from 10-11 day old embryonated hen's egg were used.

Pieces of CAM were exposed to heat inactivated virus and then incubated at 37°C. After 24 hours, the membranes were removed and washed. Then the membranes were challenged with active influenza virus. The membranes were again incubated at 37°C for an additional 48 hours after which the fluids were titrated for their hemagglutinin content.

It was found that interference had been induced in the pieces of CAM. They also found that it took 4 hours for interference to become established in the membranes and then subsequently interferon was found in the culture medium. The amount of IF detected was related to the time of preliminary incubation. At zero time, there was no IF demonstrated in the membrane nor in the fluids. At 6 hours there was relatively greater amount of IF in the membrane extracts and slightly less in the fluids. The greatest interfering activity in the fluids was found at 12 hours

and by this time it was low in the membrane. This finding suggested that most of it had been liberated into the medium.

In retrospect it is likely chat Lennet and Koprowski (1946) first observed the effects of interferon in that the virus-free fluid from minced chick embryo tissue cultures infected with 17DD high strain of yellow fever virus imparted resistance to the Venezuelan-equine-encephalitis and West Nile viruses.

Since 1957 interferon has been observed to be produced by many viruses in many different experimental systems. Most of the viruses have been shown to produce interferon as active and inactive forms. As yet it has not been shown whether Mengovirus causes the production of IF or interference with any other challenge virus. Until 1961 it was used only as a challenge virus in IF experiments. For example, Vainto et al. (1961) attempted to produce IF with West Nile virus in adult mice intracerebrally from strain BSVS (virus susceptible mice) and strain BRVR (virus resistant mice) and tested for its presence in L-cell cultures with Mengovirus as challenge. The inhibitory effect against Mengovirus was observed with brain extracts from BSVS mice only. No explanation was given for this result.

It has been established in recent studies that Mengovirus is susceptible to IF. Levy (1964) studying the mechanism of action of IF, found that Mengovirus was susceptible to IF produced by Chikungunya virus in L-cells. L-cell cultures were infected with Chikungunya virus at the multiplicity of 10 viruses per cell and incubated at 37^{o}C for 24 hours. After 24 hours of incubation the whole culture was frozen, thawed and then brought to pH 2 with hydrochloric

acid. The solution was kept at pH 2 and at 4°C for 12-18 hours to destroy the residual virus and then brought to pH 7 with sodium hydroxide.

In the experiments done with IF prepared in the foregoing manner, the cells were first treated with interferon and then infected with Mengovirus. Two effects of IF were found, the effect of interferon on cut-off of normal cell RNA and on cut-off of normal protein synthesis. Concomitant with the above effects, IF reduced the yield of Mengovirus. It was also shown that IF suppressed the viral RNA synthesis in infected cells but it was unable to prevent cell death. Guantt and Lockart (1966) studied the inhibition of the growth of Mengovirus by IF. IF was produced in L-cell subline cultures by infecting with NDV at the multiplicity of 1.0 pfu/cell. One protective unit (pu) of IF was defined as the highest dilution of IF which prevents the appearance of cytopathic effects (cpe) in Lts cultures when challenged with 10 pfu/cell of Western-equine-encephalomyelitis (WEE) virus at 12-15 hours after IF addition.

In all experiments the cells were incubated with IF for 12 hours prior to the addition of Mengovirus. Maximum inhibition of virus yields were found when 10-220 pu were used per culture. Higher doses were tried to prevent the complete cell destruction (which could not be prevented). It was also found that appearance of new virus was delayed by one hour and yield at 24 hours was decreased by 85% as compared with that of controls. Reduction in size and number of plaques were also found in cultures treated with IF.

In summary, Levy, and Guantt and Lockart in their respective experiments found that IF reduces the yield of Mengovirus.

but that it could not prevent cell death and virus multiplication completely.

Production of Interferon

IF may be induced by almost all the viruses, both DNA and RNA containing viruses, as well as rapidly multiplying viruses and also by tumour viruses.

Recently it has been found that the production of IF is stimulated not only by viruses by also by other agents. Youngner and Stinebring (1964) have reported the production of IF-like substance following the intravenous injection of live Brucella-Abortus organism in chickens. Hopps and associates (1964) have reported the production of IF-like substance with Rickettsia tsutsugamushi in primary chick embryo monolayers. Other workers have reported that nucleotides cause the production of IF-like substance in embryonated eggs and chick fibroblast cultures. Kleinschmidt, Cline and Murphy (1964) found that Statolon from Penicillnium-Stoloniforum caused the production of an IF-like substance in primary cultures of chick embryo cells. Statolon is polyanionic polysaccharide; it might provide an essential stimulus for the production of IF. Ho (1964) has found an IF-like substance in serum following the inoculation of animals with bacterial endotoxins.

Early evidence suggested that the nucleic acid component of virus triggered the production of IF but it has been shown by Isaacs, Cox and Rotem (1963) that heterologous RNA, for example mouse RNA in chick embryo cells in vitro induced the production of IF. They also found that chick RNA in chick tissue similarly induced IF production but only if it was treated with nitrous acid, which rendered it foreign to the

homologous cells by altering the pyrimidine bases.

IF production seems to be a general phenomenon in virus infected tissues. It has been found to occur in most of the animal species. Embryonic and tumour cells have also been reported to produce IF. Production of IF can be stimulated by live, incomplete, UV-inactivated and heat-inactivated viruses.

It is still not clear how IF is produced or what is the relationship of virus infection and virus induction to the formation of IF.

It is known that actinomycin D inhibits the function of DNA by combining with guanine bases on the double DNA helix. It inhibits the formation of m-RNA and hence genome-directed protein. Evidence for this factor has been shown by Ho et al. (1964). They found that if actinomycin D was added 3 hours after the adsorption of NDV in chick cultures, m-RNA was formed and IF was also produced. If added before 3 hours, m-RNA was not formed and no IF was produced.

Still another question of whether IF is a newly synthesised molecule or a slightly modified pre-existing one, is not answered. However production of IF requires the formation of new protein. This has been shown by Ho (1964). The cells were first treated with puromycin, which is a specific inhibitor of protein synthesis. IF formation was inhibited by puromycin. He concluded that synthesis of new protein is required for the production of IF.

It can be said that production of IF requires m-RNA and also some kind of new protein production. It is still not known how IF is produced inside the cell. Ho (1964) postulated another phenomenon called "priming". If inactivated virus is added to cell cultures prior to

infective virus, it will prime IF production. That is, IF is produced when none is produced in the absence of primer, or it may potentiate or increase IF production when infective virus alone is effective as an inducer. It is possible that inactive virus acting as a primer functions as an incomplete inducer by forming intracellular IF which is not released, but which can act within the cell.

Properties of Interferon

IF is a protein (Burke, 1960) because it is destroyed by proteolytic enzymes such as trypsin and pepsin. The exact molecular weight of IF is not known because different molecular weights have been given by different workers. Porterfield, Burke and Allison (1960) have determined the molecular weight of IF by measuring its rate of diffusion through agar to be about 63,000, very near to the molecular weight of hemoglobin.

Later on, Lampson, Tyrrell, Nemes and Hillman (1963) calculated that the molecular weight of IF ranged from 20,000 - 34,000 by Svedberg equation. Merigan (1964) determined the molecular weights of both chick and mouse interferons to be approximately 38,000 and 26,000 respectively, by comparing the data of G-100 Sephadex experiment with those of the experiment with marker proteins of known molecular weights. From the data reported by various workers for the molecular weights of interferons, it is impossible to state any definite molecular weight for interferon. Since interferons differ in various animal species, as do the other proteins, perhaps that is why they have different molecular weights.

IF is non-dialyzable and is stable over a range of pH from 1-10 pH

units (Lindenmann et al., 1957). Most workers have found IF to be stable or to suffer only partial loss of activity upon heating at 56°C for 30 minutes (Isaacs, 1963). However, activity is lost when preparations are heated at 76°C for one hour. Merigan (1964) found that heat-inactivation curve of highly purified mouse-produced IF was different from similar preparations of chick cell-produced IF. IF gave an UV absorption spectrum characteristic of a protein with maximum absorption at 278 mμ and minimum at 253 mμ. Lampson and co-workers (1963) have reported some of the constituents of IF: tyrosine 2.3%, tryptophan 2.6%, arginine 7.3%, lysins 11.1% and also some traces of carbohydrates. Burke (1961) reported that IF contains 2.4% of hexosamine. They also found that IF activity was destroyed by proteolytic enzymes but not by peptidases, amylase, lipase, ribonuclease, or deoxyribonuclease.

IF has been purified by many workers, but maximum purification has been obtained by Lampson and workers (1963). They purified chick IF by 4500-fold. IF is a poor antigen. It gives rise to neutralizing antibodies only after a long period of immunization (Burke and Isaacs, 1960).

Species Specificity

Interferon is effective against many kinds of viruses, but it is found that IF from one species is most active in the same species. Sutton and Tyrrell (1961) have reported that monkey IF was active in human and calf tissue, although calf IF failed to manifest antiviral activity in monkey kidney cells. The specificity therefore is not absolute for it can be active in different species or heterologous cells, but not to the same degree. Merigan (1964) however, has shown that some

preparations of IF are species specific. He showed that IF produced in mouse tissue does not inhibit the replication of viruses in chick embryo cells and conversely chick tissue-produced IF is not active in mouse cultures.

Mode of Action of Interferon

At an early stage it was clear that IF acted by rendering cells resistant to virus multiplication and did not act on extracellular virus. There is no direct interaction of virus and interferon.

IF does not affect virus adsorption or virus release from cells. It is clear that IF affects virus replication intracellularly. This was first found by Isaacs and Burke (1958) and later confirmed by Wagner (1961). IF acts at quite an early stage of viral replication. It inhibits viral RNA replication by some indirect mechanism. It also inhibits to a certain degree the cell associated virus antigens, e.g., viral hemagglutinin (Lindenmann, Burke and Isaacs, 1957). It does not affect extracellular RNA (Ho, 1961). It seems very clear from the findings that IF acts after the virus adsorption to the cell, after it has penetrated and after its protein coat has been removed but before the viral RNA is replicated.

It is not yet possible to describe the site of action of IF within the cell. Hillman (1963) stated that it seems clear that the mechanism of action of IF is most probably confined to a single cell or small group of cells rather than acting by some diffuse cellular toxic reaction.

Isaacs, Klemper and Hitchcock (1961) have promoted a series of concepts of IF action based on alteration in carbohydrate metabolism. They developed this hypothesis when they found that IF-treated cells

showed increased glycolysis, increased lactic acid production and increased oxygen consumption. The similarity between these effects and those observed when cell cultures were exposed to substances which uncouple or inhibit cellular oxidative phosphorylation, for example, dinitrophenol. From these similarities they put forward the hypothesis that IF acts by uncoupling oxidative phosphorylation. There was less formation of adenosinetriphosphate (ATP) which was needed for virus production, hence the virus replication was inhibited.

Evidence against this hypothesis has been given by a number of workers. Zemla and Schramek (1962) were able to show that IF inhibited the replication of Western-equine-encephalitis (WEE) virus which grows under anaerobic conditions. More specifically, Lampson and co-workers (1963) reported from their highly purified IF preparation that it no longer affected glycolysis and suggested that the effects of crude preparations were due to impurities.

Previously it was thought that IF does not affect cancerous and embryonic cells but it is known now that IF acts both in malignant (Cocito, DeMaeyer and DeSomer, 1962) and embryonic cells (Isaacs and Baron, 1960). This contradicts the general principle and data does not support the concept that IF acts by uncoupling oxidative phosphorylation because cancer cells and embryonic cells are believed to rely more heavily on anaerobic glycolysis than upon oxidative mechanisms. Levy, Snellbaker and Baron (1963) have presented evidence associating IF with altered RNA metabolism in virus infected cells. Cocito, DeMaeyer and DeSomer (1962) have suggested that IF acted by inhibiting the formation of a species of RNA present in virus infected cells treated with interferon.

This RNA had many of the characteristics of viral m-RNA. IF acted by inhibiting the formation of this important fraction of RNA in virus infected cells.

The concept of mechanism of action of IF has become more complicated following recent evidence that antiviral action of IF in cells may depend upon the production of protein which inhibits the viral replication. It has been shown by Taylor (1964) that actinomycin D also inhibited the action of IF. This work has been confimed by Lockart (1964) who showed that when cultures were treated with IF and then actinomycin D added after one, two and three hour intervals, an actinomycin resistant inhibition occurred which was interpreted to suggest that the longer the time between IF treatment and addition of actinomycin D, the more mediator is formed.

One current working hypothesis for the mechanism of action of IF is that in sensitive cells IF stimulates or induces the production of a new protein. This new protein inhibits the viral replication, most probably by preventing the formation of a fraction of RNA, which is necessary for viral replication. Friedman and Sonnabend (1964) also demonstrated that fluorophenylalanine, which inhibited protein synthesis, also decreased the action of IF. Glasky and associates (1964) provided another evidence that in cell-free preparations, IF inhibited a RNA synthesizing enzyme found in cells infected with RNA virus. In spite of so much evidence, the mechanism of action of IF is not clear as yet. It can be only said that IF acts by inhibiting RNA replication. It is hoped that confirmation of the work with purified IF preparations may follow in the near future. It also seems possible that IF might eventually

be used as a therapeutic agent in the control of viral infections.

Neutralization

The reaction which destroys the infectivity of the virus with virus specific antibodies is called neutralization. Unlike the intracellular action of interferon, neutralization is the result of direct extracellular combination of antibodies with virus particles. The exact mechanism of neutralization of virus by immune serum is obscure but it is known that neutralized viruses are prevented from attaching to and penetrating host cells. Lafferty and Oertalis (1963) described 3 phases of inactivation so as to understand the mechanism of neutralization or inactivation of viruses better. They pointed out that neutralization reaction proceeds in 3 stages. The first stage is called the lag phase, and detection of this phase depends upon serum concentration. If the serum concentration is too high this phase cannot be detected. Temperature is also another factor which affects this phase and can be only detected at lower temperatures than 37^{o}C (Delbecco, Vogt and Strickland, 1956).

The second stage in the neutralization reaction is one of rapid exponential inactivation. During this period the rate of inactivation is proportional to serum concentration.

The third stage is a slow rate of inactivation which is marked by the levelling of the rate versus time showing a resistant fraction of virus. The system will have completely neutralized the virus along with some resistant virus fraction. The fraction which is resistant to antibodies is either due to heterogeneity of the virus particles or due to heterogeneity of the antibodies. It was found that this was due to heterogeneity of antibody molecules.

The second type of resistant fraction was called the cell-dependent protected fraction. Lafferty (1963) used a fixed concentration of influenza virus and mixed it with an equal volume of diluted rabbit serum. In another reaction, he used fowl serum and found that there was considerable difference in the proportion of the resistant fraction obtained from each reaction. However, the reaction between virus and antibody is usually considered to be a first order reaction. The neutralization constant of this reaction can be represented by the following equation as represented by McBride (1959):

$$K = 2.3 \frac{D}{t} \log \frac{V_o}{V_t}$$

where V_o is the initial concentration of the virus at time = 0 and V_t is the concentration of the virus at time = t. D is the dilution of the serum. K is the neutralization constant. The rate of neutralization is dependent on temperature and pH which vary with different systems.

The basis of the neutralization reaction is explained by different hypotheses which were supported by electron microscope studies and by the formation of different kinds of antibodies which take part in the neutralization reaction. The hypothesis put forward by Lafferty and Oertalis (1963) is most likely to hold. According to this hypothesis, the union between virus and antibody is characterized by two stages. In the first stage the antibody molecule attaches at one of its terminal sites to the virus and sticks out like a porcupine. In the second stage the antibody molecule bends over and attaches to the virus at the other terminal site. At this stage of the union, the antibody is flattened against the surface of the virus. The antibodies thus would attach to the virus

particle by the end as outlined in the Lafferty and Oertalis hypothesis.

Action of Ribonuclease

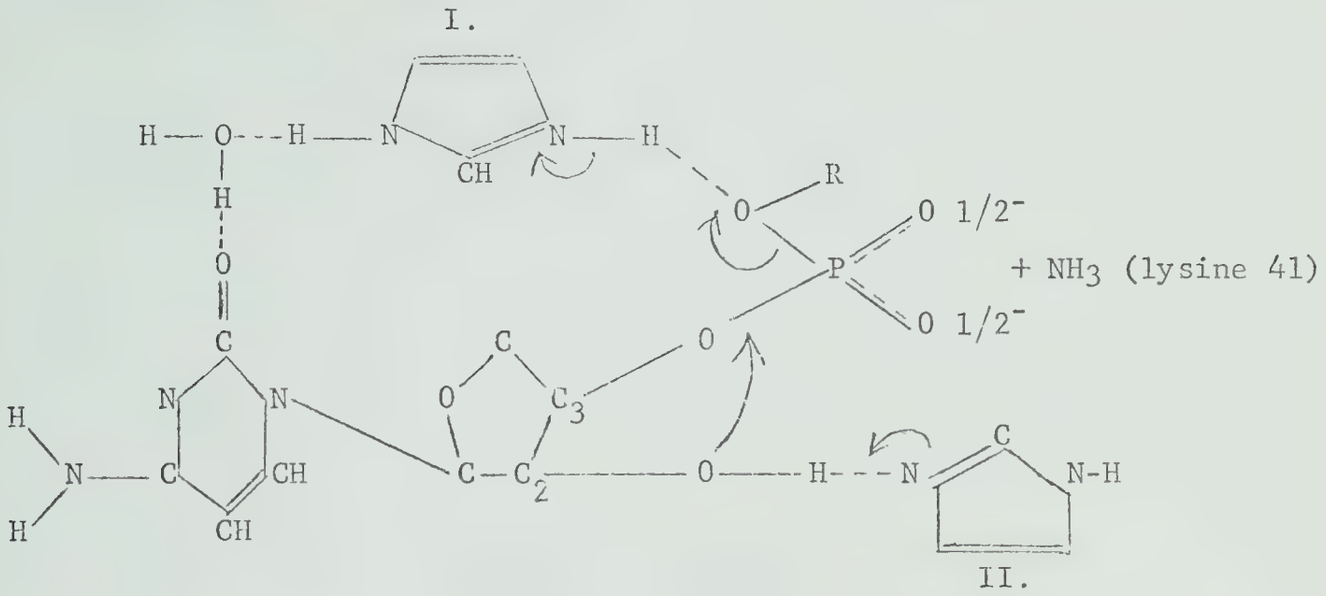
A ribonuclease is an enzyme which hydrolyzes the polynucleotide structure of ribonucleic acid into oligonucleotides or mononucleotides, thus destroying the structure of ribonucleic acid.

Most of the work on ribonuclease has been done on ribonuclease from bovine pancreas which after its action on ribonucleic acid yields the final product as nucleotide 3'-phosphates and oligonucleotides.

There is another important ribonuclease which is called phosphodiesterase and which has been separated from snake venom. When ribonucleic acid is treated with the enzyme the main products are nucleoside 5'-phosphates. Ribonuclease bovine is a protein containing 124 amino acid residues. Treatment of ribonuclease with iodoacetate at pH 5.5 produces two inactive monocarboxymethyl histidine derivatives of enzyme. Both of these derivatives are inactive; it is evident that both histidine residues must be essential for enzymic activity.

Most of the work on the action of bovine pancreatic ribonuclease has been done by Deavin, Mathias and Rabin (1966). The binding of nucleotides to ribonuclease may be investigated by spectrophotometric methods. The spectra of purine and pyrimidine in nucleotides is affected on interaction with the active site of ribonuclease. Pancreatic ribonuclease possesses two sites, one which is specific for pyrimidines and the other is specific for purines.

The enzyme substrate complex for an ester substrate is shown as follows:



The attack of the 2'-hydroxyl group is promoted by hydrogen bonding to imidazole II. Deavin, Mathias and Rabin (1966) said that model building reveals that imidazole II lies above, parallel and in partial contact with the pyrimidine ring of the substrate, but if the ester group is a purine nucleotide, the ring can be above and in contact with imidazole II. They have proposed that step one requires the interaction of one of the histidine residues in the base form with 2'-hydroxyl and the other in the acid form, with the oxygen atom binding R and P. In step two, residue in the acid form reacts with 2'-oxygen and the base form of residue binds the attacking water molecule. Mathias and Rabin (1967) have presented evidence which indicates that the active site of ribonuclease contains two histidine residues which function as an acid and a base. Deavin, Mathias and Rabin (1966) have shown that ribonuclease catalyses the hydrolysis of substrate in a two-step reaction involving the intermediate formation of a cyclic phosphate.

SOURCES OF MATERIALS

Sources of Materials

1. Amino acids: For minimum essential medium (Eagle) Microbiological Associates Inc., Bethesda, Maryland.

2. Vitamin mixture: For basal medium (Eagle) Microbiological Associates Inc., Bethesda, Maryland.

3. Fetal bovine serum: Microbiological Associates Inc., Bethesda, Maryland.

4. L-Glutamine crystalline grade II: Sigma Chemical Company, St. Louis, Missouri.

5. Penicillin G potassium: Ayerst Laboratories, Montreal, Quebec.

6. Streptomycin sulphate (Stropolin): Glaxo Allenbury's, Toronto, Canada.

7. Noble agar: Difco Lab., Detroit, Michigan.

8. Ribonucleic acid "C" grade - yeast ribonucleic acid: California Corporation for Biochemical Research, Los Angeles, California.

9. Prescription glass bottles: Dominion Glass Company, Toronto, Canada.

10. Plastic tissue culture petri dishes: Falcon Plastics, Division of B-D Laboratories Inc., Los Angeles, California.

MATERIALS AND METHODS

Materials and Methods

1. Earl's Balanced Salt Solution (EBSS)

Concentrations indicated were per liter final volume.

NaCl	6.8 gm
KCl	0.4 gm
$MgSO_4.7H_2O$	0.2 gm
$NaH_2PO_4.H_2O$	0.14 gm
$CaCl_2$	0.2 gm
Glucose	1.0 gm

0.25 ml of 2% phenol red were added.

The above solution was 10 times concentrated, it was diluted before use and 1.75 ml of 10% $NaHCO_3$ were added to each 100 cc of EBSS to bring the pH to neutrality.

2. Growth medium

To 100 cc of sterile EBSS the following substances were added:

Amino acids (Eagle) - 1 ml of 100 x concentration (2.13 gm/100 cc)

Vitamins (Eagle) - 1 ml of 100 x concentration (71 mgm/100 cc)

Glutamine - 1 ml of 200 x concentration (6 gm/100 cc)

Penicillin G potassium - 0.1 ml containing 250,000 units in water

Streptomycin sulphate - 0.1 ml of 33% of streptomycin sulphate solution (1 gm/3 cc water)

Fetal bovine serum - 5 ml

3. Nutrient agar overlay

Component (i) - Growth medium which had been made to contain double the normal concentration of amino acids, vitamins, glutamine and serum.

Component (ii) - 3% (w/v) noble agar in EBSS. Component (ii) was first liquified by autoclaving for 15 minutes. Both components were then placed in a water bath at temperature of 45°C. Before use, equal volumes of both the components were mixed together.

4. Neutral red overlay

To 95 cc of distilled water, 5 cc of 1:100 neutral red dye solution was added, then 1% noble agar (w/v) in the above solution was made. The mixture was autoclaved and kept in a water bath at 45°C.

5. Maintenance Medium

It was the growth medium without fetal bovine serum.

6. Preparation of plates for diffusion plate method

It was prepared as follows: substances were used per 100 ml of distilled water.

$MgCl_2 \cdot H_2O$	0.081 gm
$CaCl_2$	0.044 gm
Difco agar	1.500 gm
Tris	0.121 gm

pH adjusted to 8.8

The mixture was heated to melt the agar, cooled to 50°C, then 100 mg of C grade RNA was added. The mixture was poured into culture dishes and allowed to solidify.

7. Molybdic amino naphthol sulfonic acid (ANS) reagent

$(NH_4)_6MO_7O_{24}\cdot 4H_2O$ (2.5% w/v in 3N H_2SO_4) — A

1-amino-2 naphthol-4 sulfonic acid (0.25% w/v)

$NaHSO_3$ (15% w/v) — B

Na_2SO_3 (0.25% w/v)

ANS reagent was prepared by mixing 1 volume of A and 0.4 volumes of B in 8.6 volumes of distilled water.

L-cell cultures

Stock cultures of L-cells were grown as monolayers in sterile 12 oz glass prescription bottles with 10-12 ml of growth medium per bottle. The cultures were kept incubated at 37°C in a Wedco gas-flow incubator in which relative humidity was 80-90% and the carbon dioxide to air ratio was 1:20. The cultures were kept in active growth by transfering them to new bottles by scraping them with sterile rubber scrapers. For all the experiments done, cells were used from the stock cultures. Some of the cells were also kept in maintenance medium which were used at the time of need.

Mengovirus

The M-variant of Mengovirus was obtained from Dr. Colter, Department of Biochemistry, University of Alberta. Stock virus was prepared by propagating the seed virus in L-cell monolayer cultures.

L-cell cultures were prepared in sterile 3 oz glass prescription bottles. The growth medium was removed and the cells were washed with

EBSS. The cells were then infected with various appropriate dilutions of Mengovirus, using 0.2 ml of inocula of each dilution per bottle. Control cultures were treated with 0.2 ml of EBSS per bottle. The cultures were then incubated at 37°C in a Wedco gas-flow incubator for 1 hour in order to allow sufficient time for the attachment of the virus. After 1 hour the cultures were again washed with EBSS and 3.0 ml of growth medium was added in each bottle and cultures were again incubated at 37°C for 3 days. After 3 days the cultures showed signs of marked infection. Cultures were destroyed and most of the cells came off the glass surface. The control cultures were normal and growing. Fluids from infected cells were harvested and centrifuged in an International SVB centrifuge at 2500 rpm for 10 minutes to remove the gross cell debris. Virus was titrated by using the plaque assay method in L-cell monolayer cultures. The stock virus contained 1.6×10^9 plaque forming units per milliliter (pfu/ml). Aliquots of 0.2 ml of stock virus were sealed in glass ampoules and stored at -70°C in a carbon dioxide ice chest. All the experiments were carried out with this virus.

Vaccinia virus

Vaccinia virus was obtained from the Department of Microbiology, University of Rochester, N.Y. Stock virus was prepared by passing vaccinia virus twice in L-cell cultures. The L-cell cultures were prepared in the same way as described for the Mengovirus stock preparations. The cultures were infected with 0.2 ml of diluted vaccinia virus and incubated in the incubator for 2 hours at 37°C. After 2 hours the cultures were again washed with EBSS, 3.0 ml of growth medium per bottle

were added and the cultures were incubated at 37°C for 3 days. After 3 days the cultures showed confluent plaques, the fluids were harvested and stored at -60°C.

The above virus suspension was passed the second time in L-cell cultures as described above. The fluids from the infected cultures were harvested after the third day and subjected to centrifugation in an International SVB centrifuge at 2500 rpm for 10 minutes. The supernatant was pooled and titrated in L-cell monolayer cultures by the plaque assay method. The stock virus contained 1.7×10^4 pfu/ml of vaccinia virus. The stock was stored in 5.0 ml volumes in screw capped bottles at -60°C.

Newcastle disease virus (NDV)

Newcastle disease virus (NDV) was obtained from the Department of Microbiology, University of Rochester, N.Y. Stock NDV was prepared by passing the virus in chick embryo monolayer cell cultures in 60 mm sterile plastic tissue culture petri dishes. Cultures were washed with EBSS, 0.2 ml of diluted virus suspension was added in each culture and incubated at 37°C in a Wedco gas-flow incubator for 2 hours. After 2 hours the cultures were washed and 3.0 ml of growth medium was added in each culture. After 3 days the cell cultures along with the fluids were harvested and stored at -60°C. The cells were thawed and the mixture (broken cells + fluid) was centrifuged the same way as described previously. The supernatant was pooled and the virus was titrated as hemagglutinin units (HA). The virus preparation contained 256 HA/ml or approximately 2.5×10^8 virus particles/ml. The virus was stored as 3.0 ml volumes in screw capped bottles.

PART I STUDIES OF MENCOVIRUS

A. Assay of the Virus

Assay of Mengovirus was done by plaque assay method in monolayer L-cell cultures. L-cell monolayer cultures were prepared in 60 mm sterile plastic tissue culture petri dishes. The cultures were washed with EBSS after removing the growth medium. The cultures were then infected with 0.2 ml of appropriate dilution of virus having about 300 pfu per culture. Another batch of cell cultures was infected with 0.2 ml of diluted virus having approximately 30 pfu per culture. Control cultures were first treated with 0.2 ml of EBSS. The cultures were then incubated at 37°C for 1 hour in a Wedco gas-flow incubator. After 1 hour of incubation, which was given for the attachment of the virus to L-cells, the cultures were washed with EBSS to remove the unattached virus from the cultures. The cultures were then overlayed with 5.0 ml of nutrient agar overlay per culture, the preparation of which has already been described. After the agar had solidified, the cultures were incubated at 37°C for 72 hours. After 72 hours of incubation, 1.0 ml of neutral red overlay was added per culture. Preparation of this solution has also been described. The cell cultures were covered with dark paper to protect them from light and incubated at 37°C for another 8-10 hours. After this time interval the cultures were checked for plaques, which were seen grossly as clear unstained almost round areas of 2-3 mm in diameter.

B. Attachment Studies

Attachment studies of Mengovirus on L-cell cultures were made to find out optimum attachment time of Mengovirus to L-cells. The method of the experiment is similar as that used by Colter, Davies and

Campbell (1964) for the rate of attachment studies.

L-cell monolayer cultures were prepared in 60 mm plastic tissue culture petri dishes. Procedure of the experiment is the same as described previously with few variations. The cultures were infected with 0.2 ml of diluted virus having approximately 30 pfu per culture. Control cultures were treated with 0.2 ml of EBBS. The cell cultures were then incubated at 37°C in the incubator. After every 1/2 hour until 6 hours a batch of infected and control cell cultures was removed, overlayed with nutrient agar overlay and incubated for 72 hours and then stained with neutral red overlay as described in previous Experiment A. After 8-10 hours of incubation the cell cultures were checked for plaques on each culture. The plaques were counted and it was found that maximum number of viruses attached at 1 hour after infecting the cells. One hour was selected as an optimum attachment time at 37°C. Therefore for all further experiments with Mengovirus 1 hour attachment time was used for Mengovirus to L-cell monolayer cultures.

C. Studies of the Attached Virus and Unattached Virus

Monolayer L-cell cultures were prepared in 60 mm plastic tissue culture petri dishes and infected with approximately 100 pfu/0.2 ml per culture as described in the previous experiment. The control and infected cultures were incubated at 37°C for 1 hour.

After 1 hour the cultures were washed 3 times with EBSS by using 1.0 ml, 2.0 ml and 2.0 ml of solution each time per culture. The washings from each culture were pooled separately. Plaque assay was done both for attached and unattached virus particles. For unattached virus particles the monolayer L-cell cultures were infected with 0.2 ml of the

saved washings for each culture. At the end of the experiment the number of plaques were counted.

D. Growth Curve Experiments

(a) Growth curve experiment by using a low titer of the virus to infect the cultures

L-cell monolayer cultures were prepared in 1 oz sterile glass bottles. Then the growth medium was removed and the cells were washed with EBSS. The cultures were then infected with 100 virus particles per culture using 0.2 ml of appropriately diluted virus per culture. Control cultures were treated with 0.2 ml of EBSS. All the cultures were incubated at 37°C for 1 hour. After 1 hour the cultures were washed, 3 ml of growth medium was added to each culture and further incubated at 37°C. After every 9, 18 and 27 hours the fluids from the cultures were removed and saved for the titration of the extracellular virus.

(b) Titration of the extracellular virus

Titration of the extracellular virus was done by the usual plaque assay method, as already described in Experiment A. The only difference was that the cultures were infected with the appropriate dilutions of the fluids which were saved at 9 hour time intervals until 27 hours in experiment (a). Then the general method for plaque assay was followed.

PART II

1. STUDIES OF EFFECT OF HOMOLOGOUS "INTERFERON" ON MENGOVIRUS

2. INTERFERENCE STUDIES OF MENGOVIRUS WITH HOMOLOGOUS INACTIVATED VIRUS

1.A. "Interferon" Preparation by Live Mengovirus in L-cell Monolayer Cultures

L-cell monolayers were prepared in 1 oz sterile glass bottles. Cell count was done from one of the representative cultures and the approximate count was 2.5×10^6 cell/ml. The cell cultures were infected at the multiplicity of 5 p.f.u. / cell by using 0.2 ml of appropriate virus dilution. Control cultures were treated with 0.2 ml of EBSS/bottle. The cell cultures were incubated at 37°C for 1 hour. After 1 hour the culture medium was removed and washed with EBSS. 3.0 ml of fresh growth medium was added and again incubated at 37°C for 24 hours, after which the fluids from infected cell cultures were harvested and pooled. The fluids from control cultures were also harvested and pooled.

The fluids from control and infected cultures were centrifuged for 10 minutes at 2500 rpm to remove the gross cell debris. The fluids from control and infected cultures were then dialysed separately by adding 3.0 ml of fluids to each dialysis bag and hanging in the flask containing cold EBSS, which had been brought to pH 2 by using concentrated HCl. Dialysis was carried out at 6°C for 24 hours. The solution was kept stirred by a magnetic stirrer. After 24 hours the fluids were dialysed against EBSS having pH 7.2 at 6°C for 48 hours, the solution being changed with the same after 24 hours. After 48 hours the bags were punched and squeezed in a sterile flask and later transferred to sterile 3 oz bottles. Fluids were stored in a refrigerator at 6°C. Dialysed fluids from infected cell cultures were supposed to contain IF.

(a) Test for infectivity

After the acid inactivation of the virus by dialysis, the fluids were examined for any residual infectious virus particle by plaque assay method. No infectious virus was found, the virus was inactivated completely.

B. Effect of Homologous "Interferon" on the Growth of Mengovirus

L-cell monolayers were prepared in 60 mm plastic tissue culture petri dishes. Growth medium was removed and the cells were washed with EBSS. The cell cultures were then treated with 0.5 ml of "interferon" ("IF") preparation and the control cultures were treated with 0.5 ml of dialysed control culture fluids. Cell cultures were incubated at 37°C for 1 hour. After 1 hour the cells were washed and 3.0 ml of fresh EBSS were added to each culture. The cell cultures were again incubated for another 6 hours at 37°C. The EBSS was removed after 6 hours, the cultures were washed, challenged with approximately 50 pfu of Mengovirus per culture and the usual plaque assay method was followed as described previously. At the end of the experiment, both the "IF"-treated cultures and the control cultures were checked for the plaques.

C. Effect of "Interferon" on the Growth of Mengovirus after Giving Different Time Intervals of Incubation for the Interferon-Treated Cell Cultures

The experimental procedure was the same as described in Experiment B, the only difference being that 6 hours, 12 hours, 18 hours and 24 hours of incubation at 37°C were given for the "IF"-treated cell cultures and control cultures.

After each above mentioned time interval, a batch of cell cultures was removed, challenged with 50 pfu of Mengovirus as described previously and the usual plaque assay method was followed.

D. Yield of Mengovirus With Time After Treating the Cell Cultures With "Interferon"

L-cell monolayers were treated with "IF" as described in Experiment B.

Then these "IF"-treated cell cultures and control cultures were challenged with approximately 100 pfu of Mengovirus per culture. The cultures were incubated at 37°C for 1 hour, after which the cultures were washed, 5.0 ml of growth medium was added to each culture and then the cultures were again incubated at 37°C. Fluids from "IF"-treated cultures as well as from control cultures were withdrawn every 9 hours, 18 hours and 27 hours and stored at -60°C for the titration of the virus. Another plaque assay experiment was done for the titration of the yielded virus by infecting the monolayer L-cell cultures with the appropriate dilutions of the virus.

E. Studies of the Unattached Mengovirus from the "Interferon" Pre-treated L-cell Monolayer Cultures

Monolayer of L-cell cultures were treated with "IF" as described in previous experiments. The "IF"-treated cells and the control cell cultures were then challenged with 100 pfu of Mengovirus per culture and incubated at 37°C for 1 hour. Then the cultures were washed 3 times with 2.0 ml, 2.0 ml and 1.0 ml of EBSS per culture. The washings were saved for the plaque assay of unattached virus. Washings were pooled separately from each culture. The usual plaque assay was done to find out the unattached virus from "IF"-treated and control cell cultures.

F. Studies with the Partially Purified "Interferon"

(a) Partial purification of interferon

The method for the partial purification of "IF" was the same as that followed by A. Davies (1964). 15 ml of "IF" preparation was treated with enough of 5% trichloroacetic acid (TCA) at 0°C to form a precipitate.

The whole mixture was then centrifuged in a Servall refrigerated automatic centrifuge at 0°C for 15 minutes at 10,000 rpm. After centrifugation the precipitate settled down at the bottom and the TCA formed a clear layer at the top. The supernatant was poured off and the precipitate was supposed to contain "IF" along with other proteins. The precipitate was dissolved in 2.0 ml of 9:1 ethanol-4 mM HCl at -5°C. The pH of the solution was brought to 6.0 by EBSS. The solution was supposed to contain partially purified "IF". Dialysed control fluids were also treated the same way.

(b) Effect of partially purified "Interferon" on the growth of Mengovirus.

The experiment was done exactly the same as Experiment B, the only difference being that the cell cultures in this experiment were treated with partially purified "IF" instead of "IF" and challenged with approximately 100 pfu per culture of Mengovirus. The rest of the procedure was exactly the same.

G. Studies with Extracellular and Intracellular "Interferon"

(a) Preparation of extracellular and intracellular "interferon" by the live Mengovirus in L-cell monolayer cultures

L-cell monolayers were prepared in 1 oz glass bottles. The growth medium was removed, the cultures were washed with EBSS and infected with 0.2 ml of virus dilution per culture having 300 virus particles. Control cultures were treated with 0.2 ml of EBSS per culture. The cultures were incubated for 1 hour at 37°C, after which the cultures were washed, 3.0 ml of growth medium was added to each culture and further incubated for 18 hours. Fluids from infected cell cultures and control cultures were pooled separately and respective

cell cultures were frozen.

Extracellular "IF" was the "IF" which was released into the medium by the cells and which was prepared from pooled fluids by dialysis, the same way as described in Experiment A. After dialysis, the fluids were collected in screw capped bottles and stored in a refrigerator at $6^{o}C$.

Intracellular "IF" was the "IF" which was present in the cells. It was prepared by suspending the cells in growth medium and breaking the cells by the sonic oscillator. The material was then centrifuged at 2500 rpm for 10 minutes in an International SVB centrifuge. The supernatant was then dialysed as described in Experiment A. The preparations from control cell cultures and infected cell cultures were then stored in screw capped bottles in the refrigerator.

(b) Test for infectivity

Both the preparations were tested by the usual plaque assay method for any residual infectious virus in the preparation. No infectious virus was found.

(c) Effect of extracellular and intracellular Mengovirus "interferon" on the growth of homologous virus

The experiment was done exactly the same way as described in Experiment B, only the cultures were treated with extracellular and intracellular "IF" and control cultures were treated with extracellular and intracellular control fluids.

2. A. Inactivation of Mengovirus at 37°C and Interference Studies Between Heat-inactivated Virus and Infectious Mengovirus

(a) Inactivation of Mengovirus at 37°C

Mengovirus having the titer of 1.6×10^9 pfu/ml was diluted to 1.6×10^5 pfu/ml in EBSS having pH 7.2. The virus suspension was placed in a water bath at 37°C. Samples of 1.0 ml were taken every 2 hours starting from 0 hour until 56 hours. Each sample was stored at -60°C for the titration of the virus by plaque assay method. By the plaque assay method survival of pfu/ml with each time of inactivation was calculated. A curve of inactivation of the virus with the time at 37°C was established.

(b) Inactivation of high titer of Mengovirus at 37°C

An experiment was done to inactivate the high titer of mengovirus to be used in interference experiments. 5.0 ml of Mengovirus having titer of 1.6×10^8 pfu/ml was placed in a water bath at 37°C. Some of the fluids from control cultures were also placed. The virus was inactivated after 297 hours. The preparation was stored at -60°C and examined for its infectivity by the usual plaque assay method. No infectious virus was found.

(c) Interference studies between heat-inactivated Mengo and infectious Mengovirus in L-cell cultures

Monolayer of L-cell cultures were grown in 60 mm plastic tissue culture petri dishes. The cultures were washed with EBSS and treated with approximately 6 heat-inactivated virus particles per cell by using 0.4 ml of heat-inactivated virus preparation per culture. The control cultures were treated with 0.4 ml heat-treated control fluids per culture and all the cultures were incubated at 37°C for 1 hour.

After 1 hour the cultures were washed, 3.0 ml of fresh EBSS was added in each culture and incubated for 6 hours at 37°C, after which the cultures were again washed and challenged with 100 pfu of Mengovirus per culture, and then the usual plaque assay method was followed.

B. Inactivation of Mengovirus by Ultraviolet Light. Interference Studies Between Ultraviolet Light Inactivated Virus and Infectious Mengovirus

(a) Inactivation of Mengovirus by ultraviolet light

5.0 ml of Mengovirus having 1.6×10^4 pfu/ml at pH 7.2 was exposed to the irradiation of ultraviolet light (UV light) in 10 mm glass culture plates. Samples from the virus preparation was withdrawn after every 15 seconds starting from 0 time to 60 seconds. The samples of the inactivated virus were stored at -60°C. Samples of the inactivated virus were titrated by the usual plaque assay method. It was found by this method that all the virus was inactivated in 60 seconds.

(b) Inactivation of high titer of Mengovirus by ultraviolet light

5.0 ml of 1.6×10^8 pfu/ml of Mengovirus was inactivated after 255 seconds of exposure to UV light. Fluids from control cultures were also treated the same way.

(c) Interference studies between ultraviolet light inactivated Mengovirus and infectious Mengovirus in monolayer L-cell cultures

The procedure for the interference studies between UV-inactivated Mengovirus and infectious Mengovirus was similar to those of heat-inactivated virus except that approximately 6 UV-inactivated virus perticles per cell were used instead of heat-inactivated viruses. Control cultures were treated with UV-treated control fluids.

PART III INTERFERENCE STUDIES BETWEEN LIVE NEWCASTLE DISEASE VIRUS AND MENGOVIRUS. EFFECT OF NEWCASTLE DISEASE VIRUS INTERFERON ON THE GROWTH OF MENGOVIRUS IN MONOLAYER L-CELL CULTURES.

A. Interference Between Live Newcastle Disease Virus and Mengo-Virus in L-cell Cultures

L-cell monolayer cultures in 60 mm plastic petri tissue culture dishes were infected with 0.5 ml of NDV per culture with 128 HA units, having approximately 13-12 virus particles per cell. The control cultures were treated with 0.5 ml of EBSS of culture, and the cultures were incubated at 37°C for 2 hours. After 2 hours the cell cultures were washed with EBSS, 3.0 ml of EBSS were added to each culture and incubated for another 6 hours at 37°C. The cell cultures were again washed with EBSS, challenged with 100 pfu of Mengovirus per culture and the usual plaque assay method was followed.

B. Studies of the Unattached Mengovirus on Monolayer L-cell Cultures Infected with Newcastle Disease Virus

The procedure of the experiment was the same as followed for the studies of live NDV and Mengovirus in L-cell cultures (Experiment A) except that the Mengovirus challenged cultures were washed with EBSS by using first 1.0 ml and then 2.0 ml. The washings were pooled and saved and stored at -60°C. Washings were titrated by the usual plaque assay method to find out the attached and unattached virus both from control cultures and NDV-infected cultures.

C. Preparation of Interferon by Live Newcastle Disease Virus in L-cell Monolayer Cultures and its Effect on the Growth of Mengovirus

(a) Preparation of interferon by Newcastle disease virus in L-cell Monolayer Cultures

L-cell monolayers were grown in 4 oz sterile glass bottles. The growth medium was removed, the cultures were washed with EBSS and then

infected with 0.4 ml of appropriate dilution of NDV having about 100 HA units per culture. The control cultures were treated with 0.4 ml of EBSS. The cell cultures were incubated at 37°C for 2 hours, after which the cultures were again washed with EBSS and 4.0 ml of growth medium was added to each culture. The cell cultures were again incubated at 37°C for 96 hours after which the fluids from infected cultures and control cultures were harvested separately.

The fluids were then dialysed the same way as described in Experiment A for the IF production by live Mengovirus in L-cells. These fluids contain IF and they were stored in the refrigerator in screw capped bottles.

(b) Effect of Newcastle disease virus interferon on the growth of Mengovirus in monolayer L-cell cultures after 6 hours of incubation

Monolayer of L-cell cultures were prepared in 60 mm plastic tissue culture petri dishes. The growth medium was removed, cell cultures were washed with EBSS and treated with 0.5 ml of IF per culture. The control cultures were treated with 0.5 ml of dialysed control culture fluids. The cell cultures were incubated at 37°C for 2 hours, after which the fluids were washed with EBSS, 3.0 ml of EBSS were added to each culture and the cell cultures were incubated at 37°C for 6 hours. After 6 hours incubation the cell cultures were again washed and challenged with approximately 100 pfu of Mengovirus per culture and the rest of the procedure was the usual plaque assay method for Mengovirus as described in previous experiments.

(c) Effect of NDV IF on the growth of Mengovirus by giving 24 hours of incubation for interferon pretreated cell monolayer cultures

Experimental procedure was the same as described in the previous experiment done on the effect of IF on the growth of the Mengovirus except that 24 hours of incubation at 37°C were given for IF-treated cell cultures prior to the challenge with Mengovirus and after giving 2 hour incubation for the absorption of the IF to L-cell cultures. Then the cultures were challenged with approximately 100 pfu of Mengovirus per culture and the usual plaque assay method was followed.

(d) Effect of varying the doses of Newcastle disease virus interferon on the growth of Mengovirus in monolayer L-cell cultures

The procedure for this experiment was the same as that of the experiment done to see the effect of NDV IF on the growth of Mengovirus, except that IF here was used in doses of 0.5 ml and 1.0 ml instead of only 0.5 ml as used in the other experiment. The rest of the procedure followed was exactly the same.

PART IV EFFECT OF MENGOVIRUS "INTERFERON" AND PARTIALLY PURIFIED INTERFERON ON THE GROWTH OF VACCINIA VIRUS IN L-CELL CULTURES

A. Effect of Mengovirus "Interferon" on the Growth of Vaccinia Virus in L-cell Cultures

Monolayer of L-cell cultures were prepared in 60 mm plastic tissue culture petri dishes. The growth medium was removed; the cultures were washed and treated with 0.5 ml of "IF". The control cultures were treated with 0.5 ml of EBSS and the cultures were incubated at 37°C for 1 hour. The cultures were again washed with EBSS, 3.0 ml of EBSS was added to each culture and incubated for another 6 hours at 37°C. After this the cultures were again washed, challenged with 100 pfu of vaccinia virus per culture, and incubated at 37°C for 2-1/2 hours. The usual plaque assay method was followed similar to mengo virus plaque assay.

B. Effect of Partially Purified "Interferon" on the Growth of Vaccinia Virus

The procedure of this experiment was the same as described in previous experiments, except that partially purified "IF" was used instead of "IF".

PART V EXTRACTION OF INFECTIOUS RIBONUCLEIC ACID FROM L-CELLS INFECTED WITH MENGOVIRUS

A. Extraction of Infectious Ribonucleic (IRNA) Acid From L-cells Infected With Mengovirus

The method followed was the same as that used by Tobey (1964). L-cell monolayer cultures were prepared in 12 oz sterile glass bottles. The cell cultures were washed and infected with 1.6 x 10^9 pfu/ml/culture. The control cultures were treated with 1 ml EBSS per culture. The cultures were incubated at 37°C for 1 hour. After 1 hour the cultures were washed, 4.0 ml growth medium was put on per culture and cultures were incubated at 37°C for 6 hours.

After 6 hours of infection, equal amounts of .14 M sodium chloride (NaCl) were added to the cell cultures. Cell cultures were then scraped from the glass surface and centrifuged at 2500 rpm for 10 minutes. The pellet of cells was frozen immediately.

The cells were then thawed and frozen 3 times to break the cells. To the thawed cells 5% sodium dodecyl sulfate (SDS) was added to make it 0.5%. An equal amount of water saturated phenol was added at 60°C. The suspension was continuously shaken in a water bath having 60°C temperature for 3 minutes, then immersed in a -15°C ice salt bath for 30 seconds. Aqueous and phenol layers were separated by centrifugation in a Servall automatic centrifuge at 4°C, at 6000 rpm for 15 minutes. The aqueous layer was removed carefully, equal volumes of ether were added and again centrifuged at 4°C, at 6000 rpm for 15 minutes. The top ether layer was removed and discarded. To the aqueous layer, cold ethanol was added in 4-5 volumes, and the precipitate was centrifuged at 6000 rpm for 1/2 hour at 4°C. The precipitate having IRNA settled at the bottom, ethanol was discarded and the precipitate resuspended in TM buffer, [5 x 10^{-3} $MgCl_2$ and 0.01 M Tris pH 7.2].

This preparation of IRNA was tested in a spectrophotometer, the control RNA (CRNA) preparation and infectious ribonucleic acid (IRNA) preparation gave similar peaks at 269 mμ. The preparations of CRNA and IRNA were both stored at -60°C. The IRNA preparation was titrated by the usual plaque assay method as used for Mengovirus and the titer for IRNA was 430 pfu/ml.

PART VI PREPARATION OF RABBIT ANTI-MENGOVIRUS SERUM, NORMAL RABBIT SERUM, GAMMA GLOBULIN FROM NORMAL SERUM AND GAMMA GLOBULIN FROM ANTI-SERUM

A. Preparation of Rabbit Anti-Mengovirus Serum (RAMS)

Three rabbits were injected with 1.6 x 10^8 pfu of Mengovirus into the auricular vein of each rabbit. After 10 days another injection was given to each rabbit and after the same time interval one more was given to each rabbit. Then after two weeks the rabbits were bled by approaching the external carotid artery and puncturing it. The blood was collected in glass cylinders. The blood was kept in the refrigerator overnight and the next day serum from the top was removed. The RAMS was centrifuged at 2500 rpm for 10 minutes to remove some blood clots and tissue debris. The RAMS was filtered by using .45 μmillipore filter, collected in sterile screw capped bottles and stored in the refrigerator.

B. Preparation of Normal Rabbit Serum (RS)

Normal rabbit serum was prepared by the same method as mentioned above, the only difference was this time the normal rabbit was bled and then the same procedure was followed as explained previously.

C. Preparation of the Normal Gamma Globulin (from RS) and of Immune Gamma Globulin (from RAMS)

Gamma globulin (γ-globulin) was separated from the rest of the serum proteins by using a simple chromatographic method as described by Leby and Sober (1960).

The anion exchanger, diethylaminoethyl cellulose (DEAE) was washed 3 times with 0.5 N NaOH to remove colour and to regenerate the column. It was suspended in water and washed to neutrality. The slurry was adjusted to pH 6.3 by adding 0.2 M of sodium dihydrophosphate

(NaH_2PO_4) and washed several times with 0.0175 M NaH_2PO_4 buffer at pH 6.3.

For 2 ml of the dialysed serum, a column of DEAE cellulose was prepared of about 1 cm in diameter and 5-6 cm in height using ordinary natural gas pressure. The column was then washed by using 50 ml of 0.0175 M NaH_2PO_4 buffer. The RAMS and RS both were dialysed separately against 0.0175 M NaH_2PO_4 having pH 6.3 at $6^{o}C$ for 24 hours.

Two separate columns were used for the separation of immune γ-globulin and normal γ-globulin.

With the liquid level of the buffer just at top of the adsorbent 2.0 ml of each type of serum was carefully added at the top of separate DEAE columns. For 2.0 ml of serum about 6.0 ml of buffer was added to remove complete γ-globulin from the serum. Fractions of 2.0 ml each were collected and kept in the refrigerator. Fractions collected from RAMS and RS were tested by electrophoresis to determine whether the gamma globulin had been separated or not.

PART VII ELECTROPHORESIS

Electrophoresis

The electrophoresis chamber was filled with 500 ml of Gelman HR buffer. The fluid was leveled by tilting the chamber front to back, and the chamber was covered. Four cellulose acetate III strips were soaked in buffer for 10 minutes before using. After 10 minutes the strips were placed between 2 sheets of absorbent blotter paper to absorb excess buffer. Then with the applicator the samples of RAMS, immune γ-globulin, RS and normal γ-globulin were applied to each strip approximately 2 inches away from one end of the strip, at right angles to the margin. 6λ of sample was used in each case and the sample was deposited as a straight line by holding the applicator vertically and pressed firmly against the strip. The strips were then placed carefully in the chamber and tensioned by "magna" strips by placing those in the right position. The lid of the chamber was closed tightly and the power supply was turned on. Electrophoresis was carried out using about 1 ma per strip at 300 volts for 45 minutes.

At the end of electrophoresis the strips were taken out, held horizontally and placed in a tray containing Ponceau S stain for 7 minutes. After than, the strips were washed 1 minute in each of 3-4 trays of 5% acetic acid. Strips of RAMS and RS showed different bands for different serum proteins, in the order of gamma globulin towards the line of origin where the sample applied, then beta away from the origin, alpha and then albulin farthest away from the starting line. The strips, which were only treated with immune γ-globulin, normal γ-globulin only showed one band of gamma globulin along with the line of origin where the sample was applied.

PART VIII EFFECT OF RABBIT ANTI-MENGOVIRUS SERUM, IMMUNE γ-GLOBULIN, RABBIT NORMAL SERUM AND NORMAL γ-GLOBULIN ON MENGOVIRUS IN L-CELL MONOLAYER CULTURES

A. Effect of Rabbit Anti-Mengovirus Serum and Immune γ-Globulin on Mengovirus in L-cell Monolayer Cultures

Monolayer of L-cell cultures were prepared in 60 mm plastic tissue culture petri dishes. The cell cultures were washed and treated with a mixture of 0.2 ml of Mengovirus having approximately 30 virus particles and 0.2 ml of RAMS per culture. This mixture had already been kept in 37°C water bath for 1 hour prior to use on the cell cultures. Another batch of cultures was treated with a mixture of 0.2 ml of Mengovirus having 33 pfu and 0,2 ml of immune γ-globulin per culture. This mixture was also incubated at 37°C for 1 hour in a water bath.

Control cultures were treated with a mixture of 0.2 ml of virus and 0.2 ml of EBSS. All the cultures were incubated at 37°C for 1 hour. After 1 hour the cultures were overlayed with nutrient agar overlay and incubated for another 72 hours at 37°C and then stained with neutral red overlay and checked for plaques after 8-10 hours.

B. Effect of Rabbit Normal Serum and Normal γ-Globulin on Mengovirus in L-cell Monolayer Cultures

The experimental procedure was the same as described for the RAMS, and immune γ-globulin effect on Mengovirus with the only difference being that RS and normal γ-globulin were used instead of RAMS and immune γ-globulin.

PART IX EFFECT OF RABBIT ANTI-MENGOVIRUS SERUM, IMMUNE γ-GLOBULIN, RABBIT NORMAL SERUM AND NORMAL γ-GLOBULIN ON MENGO INFECTIOUS RIBONUCLEIC ACID IN L-CELL MONOLAYER CULTURES

A. Effect of Rabbit Anti-Mengovirus Serum and Immune γ-Globulin on Mengovirus Infectious Nucleic Acid in L-Cell Monolayer Cultures

The experimental procedures in this experiment were the same as described in Experiment 8, Part A, except that a mixture of 0.2 of IRNA having approximately 30 pfu and of 0.2 ml RAMS were used instead of Mengovirus mixture with RAMS. Similarly a batch of cultures was treated with a mixture of 0.2 ml of IRNA having 30 pfu and 0.2 ml of immune globulin instead of a mixture of Mengovirus and immune globulin. The rest of the procedure was the same.

B. Effect of Rabbit Normal Serum and Normal γ-Globulin on Mengovirus Infectious Ribonucleic Acid

The experimental procedure was the same as in Experiment 9, Part A, with the difference that RS was used instead of RAMS and normal γ-globulin was used instead of immune γ-globulin.

PART X DEMONSTRATION OF NUCLEASE ACTIVITY IN RABBIT ANTI-MENGO-VIRUS SERUM, RABBIT NORMAL SERUM, IMMUNE γ-GLOBULIN AND NORMAL γ-GLOBULIN BY DIFFUSION PLATE METHOD

A. Demonstration of Ribonuclease Activity by Diffusion Plate Method

The method used was that reported by Berry and Cambell (1965). Diffusion assay plates were prepared, antibiotic assay cups were slightly embedded in the agar surface. 0.2 ml aliquots of RAMS, RS, immune gamma globulin, normal gamma globulin were poured into each assay cup on separate plates. The plates were then gently put into the incubator at 37°C for 16 hours. After 16 hours the plates were flooded with 10% TCA for 15 minutes. After that the plates were flooded with molybdic aminonaphthol sulphuric acid reagent. Nuclease activity in all the plates resulted in the appearance of clear, sharply defined zones in blue-white opaque background.

B. Diffusion Plate Method from Cellulose Acetate Strips

Samples of RAMS, RS, immune γ-globulin and normal γ-globulin were subjected to electrophoresis using Gelman electrophoresis apparatus. At the end of electrophoresis, the strips were cut horizontally into two halves. One half of the strip was stained in Ponceau S stain, the other half was dried under a hair dryer and placed on diffusion plates and incubated. After 16 hours of incubation and development the clear areas were seen at the same distance from the line of origin as the bands were from the line of origin. Clear areas were clearly visible in the plate with RAMS strip. The others showed very faint areas of clearance.

PART XI SPECIFIC EFFECT OF SERUM ENZYMES ON PURIFIED TRITIUM LABELLED MENGOVIRUS INFECTIOUS RIBONUCLEIC ACID

Specific Effect of Serum Enzymes on Purified Tritium Labelled Mengo-Virus Infectious Ribonucleic Acid

This experiment was done with the help of the Department of Biochemistry, University of Alberta. Purified Mengovirus infectious ribonucleic acid which had a molecular weight of 1.7×10^6, an optical density of 1.5 at 260 mμ and labelled with tritium. It had a specific activity of 17×10^6 counts per minute per milliliter. Each incubation mixture contained 0.1 ml of labelled IRNA with 0.2 ml of RAMS, RS, γ-globulin prepared from RAMS or γ-globulin prepared from RS and 0.1 ml of Tris buffer at pH 8.8. Each mixture was incubated at $37^{\circ}C$ for 15 minutes. The incubated and unincubated controls contained no serum or γ-globulin. After 15 minutes the products were layered onto a 5%-20% sucrose gradient and then subjected to centrifugation at 35000 rpm for 3 hours in an SW 39 head in a Spinco Model L. At the end of centrifugation the distribution of radioactivity was determined.

RESULTS

Results

A. Plaque Assay of Mengovirus

After the development of plaques it was found that it was very hard to count plaques in the cultures which were infected with approximately 300 pfu per culture, whereas in the cultures infected with approximately 30 pfu per culture, the plaques were seen as distinct small rounded, clear areas surrounded by dark red stained areas. The plaques were about 2-3 mm in diameter.

B. Optimum Attachment Time of Mengovirus to L-Cells

This experiment was done by the plaque assay method to determine the optimum attachment time of Mengovirus to L-cells. The results are summarized in Table I and Fig. 1. It was found from these studies that optimum attachment time for Mengovirus to L-cells was at 1 hour. After this time there was not much increase or decrease in the number of virus particles attached to L-cells.

C. Studies of the Attached and Unattached Mengovirus on L-Cell Monolayer Cultures

The cell cultures were infected with approximately 100 pfu per culture of Mengovirus. At the end of an attachment time of one hour, it was found that on the average, approximately 85% of the virus inoculum became attached to and formed plaques on L-cell monolayers. The remaining 15% of the inoculum was found in the post attachment cell washings. Detailed results from all the cultures are summarized in Table II.

TABLE I. Attachment of Mengovirus on L-Cell Monolayer Cultures

Inoculum approximately 30 pfu/culture

Attachment Time Hours	*Mean Plaques/Culture
1/2	8.0
1	16.5
1-1/2	11.2
2	11.2
2-1/2	17.0
3	10.0
3-1/2	14.0
4	11.2
4-1/2	15.2
5	9.7
5-1/2	13.5
6	14.0

*Mean is the average of the plaques from four cultures

ATTACHMENT OF MENGOVIRUS ON L-CELL MONOLAYER CULTURE (Data From Table 1)

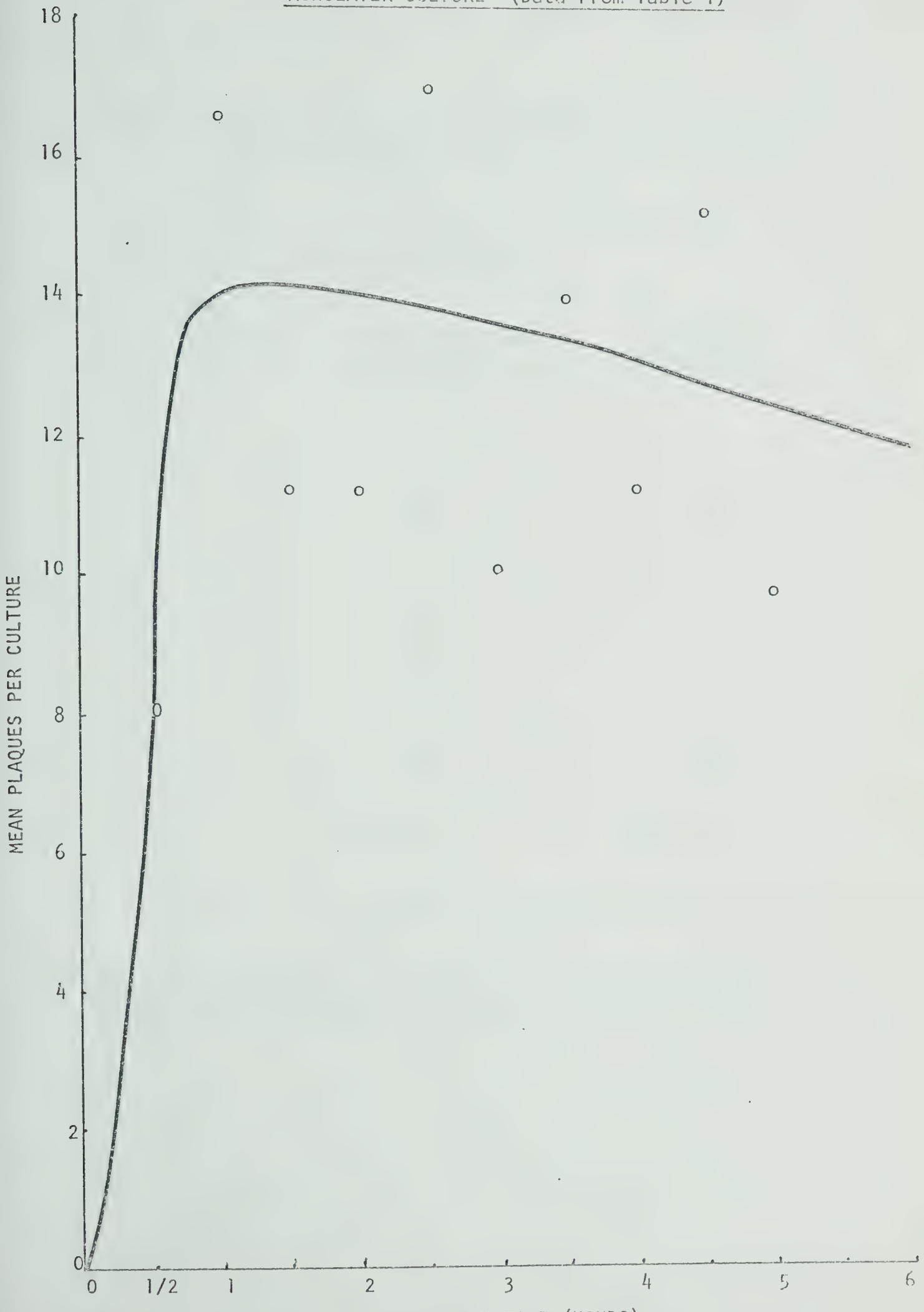


Fig. 1

TABLE II. Studies of the Attached and Unattached Mengovirus on Monolayer L-Cell Cultures

Inoculum approximately 100 pfu/culture

Culture Number	0.2 ml Containing Plaques/Culture	*Unattached Virus/Culture
1	90	25
2	95	0
3	88	0
4	70	50
5	92	0
6	90	25
7	87	0
8	72	25
	Mean: 85	Mean: 15

*For unattached virus, inoculum was 0.2 ml/culture from 5.0 ml of saved washing from each corresponding experimental culture.

D. Growth Curve Experiments

Monolayers of L-cells were infected with a low titre of Mengovirus virus as described in Materials and Methods. The yield of the virus was measured every 9, 18 and 27 hours from low titre infected cells. The results were summarized in Table III and Fig. 2.

E. Effect of Homologous "Interferon" on the Growth of Mengovirus

IF from L-cells were prepared following the infection of the cells with Mengovirus. The method has been described in Materials and Methods. The results are summarized in Table IV. It is clear from the results that the "IF" prepared from L-cells following interaction with Mengovirus did not have any effect on the growth of the Mengovirus, as the control cultures and "IF"-treated cultures showed about the same number of plaques per culture. Then another experiment was done by giving various time intervals of incubation (6,12,18 and 24 hours) of cell cultures with "IF", with the idea that a greater time of incubation might change the results. The results are shown in Table V. In this experiment, as before, "IF" did not show any effect on the growth of Mengovirus.

In yet another experiment, the yield of Mengovirus was measured after pretreating the cell cultures with Mengovirus "IF". The yield of virus from L-cell cultures was measured after every 9 hours, 18 hours and 27 hours. The results were shown in Table VI and Fig. 3. These results also showed that "IF" did not have any effect on the yield of Mengovirus in L-cells. The results of control cultures and "IF"-treated cultures were comparable; the yield of the virus was constantly increasing after every 9 hours in both the cultures.

TABLE III. Extracellular Yield of Mengovirus From L-Cell Cultures Infected with a Low Titre Inoculum

Inoculum approximately 100 pfu/culture

Hours (Post-infection)	*pfu/ml
9	1.4×10^5
18	3.0×10^6
27	4.1×10^6

*Represents the mean of four determinations.

EXTRA-CELLULAR YIELD OF MENGOVIRUS FROM L-CELL MONOLAYER CULTURES INFECTED WITH A LOW TITRE INOCULUM (Data From Table III)

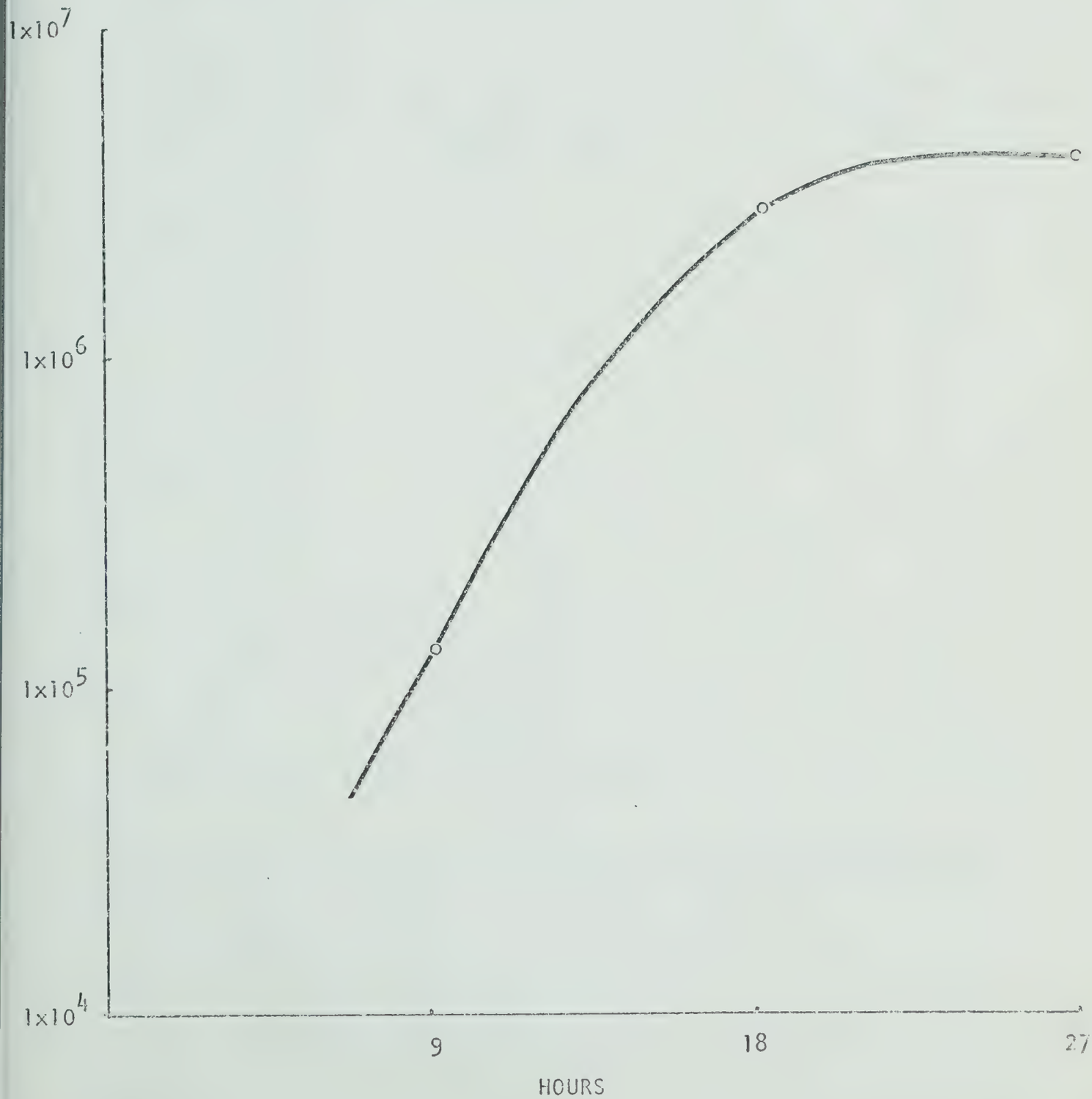


Fig. 2

TABLE IV. Effect of Mengovirus "Interferon" on the Growth of Homologous Virus in L-Cell Monolayer Cultures

*Experimental Cultures treated with 0.5 ml of "IF"
*Control cultures treated with 0.5 ml of control fluids
Challenge inoculum approximately 50 pfu/culture

Culture Number	Plaque Count on Experimental Cultures Plaques/Culture	Plaque Count on Control Cultures Plaques/Culture
1	45	45
2	50	42
3	47	48
4	42	54
5	46	51
6	52	50
7	36	41
8	46	50
	Mean: 45	Mean: 47

*For details of preparation see section on Materials and Methods.

TABLE V. Effect of Mengovirus "Interferon" on the Growth of Homologous Virus After Various Times After Incubation

*Experimental cultures treated with 0.5 ml of "IF"

*Control cultures treated with 0.5 ml of control fluids

Challenge inoculum approximately 50 pfu/culture

Time of Preincubation with IF (Hours)	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
6	40	30
	50	70
	65	45
	40	55
	Mean: 49	Mean: 50
12	66	50
	60	55
	40	67
	45	50
	Mean: 53	Mean: 55
18	70	56
	60	42
	55	61
	40	62
	Mean: 56	Mean: 55
24	40	53
	52	68
	63	41
	50	51
	Mean: 51	Mean: 53

*For details of preparation see section on Materials and Methods.

TABLE VI. Yield of Mengovirus From L-Cell Monolayer Cultures After 9 Hour Intervals, the Cultures Pretreated with Homologous "Interferon"

Experimental cultures treated with 0.5 ml of "IF"

Control cultures treated with 0.5 ml of control fluids

Challenge inoculum approximately 100 pfu/culture

Time of the Yield of Virus (Hours)	*pfu/ml in Experimental Cultures	*pfu/ml in Control Cultures
9	2.7×10^4	2.3×10^4
18	1.3×10^5	1.7×10^5
27	2.3×10^6	3.5×10^6

*Represents the mean of four determinations.

COMPARATIVE EFFECT OF MENGOVIRUS "INTERFERON"
ON YIELD OF MENGO VIRUS (Data From Table VI)

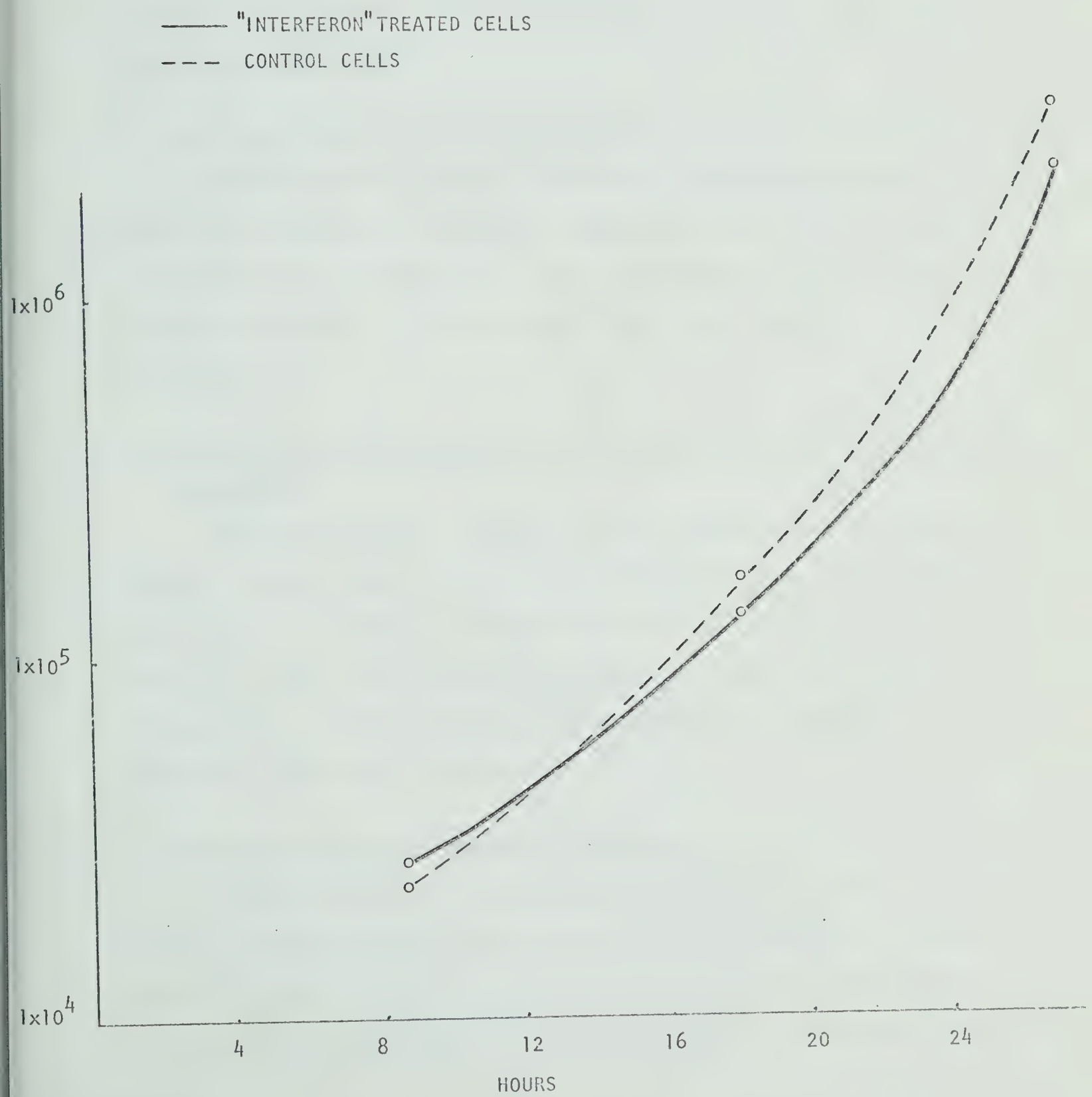


Fig. 3

Studies were also done on the attachment of Mengovirus to L-cells after pretreating the cell cultures with Mengovirus IF. It was found from the results that there was no measurable interference activity at the attachment site as almost the same number of viruses attached to the control cultures and "IF"-treated cultures. Results are summarized in Table VII.

F. Effect of Partially Purified "Interferon" on the Growth of Mengovirus

Mengovirus "IF" was partially purified by the method described in Materials and Methods. The effect of partially purified "IF" was measured in L-cell cultures on Mengovirus. Even the partially purified "IF" did not show any effect on the Mengovirus. Results are tabulated in Table VIII.

G. Effect of Extracellular and Intracellular "Interferon" on the Growth of Mengovirus

Extracellular and intracellular "IF" were prepared from the fluids and cell cultures respectively. The experiments were performed with these "interferons" as described in Materials and Methods. The results are tabulated in Table IX. Extracellular and intracellular "interferons" did not show any effect on the growth of Mengovirus as found from the comparison with control cultures.

H. The Rate of Heat-Inactivation of Mengovirus

Before the subsequent experiments, which were concerned with the ability of heat-inactivated virus to interfere with the growth of Mengovirus in L-cells, it was necessary to determine the rate of inactivation of Mengovirus at 37°C and at pH 7.2. It was found that approximately it

TABLE VII. Studies of Unattached Mengovirus From "Interferon" Treated L-Cell Monolayer Cultures

Inoculum - 0.2 ml/culture from 5 ml of saved washings.

Culture Number	Unattached Virus from IF-Treated Cultures Plaques/Culture	*Unattached Virus from Control Cultures Plaques/Culture
1	1	2
2	2	1
3	None	2
4	None	None

*For unattached virus, inoculation was 0.2 ml/culture from 3.0 ml of saved washings from each corresponding experimental culture.

TABLE VIII. Effect of Partially Purified Mengovirus "Interferon" on the Growth of Homologous Virus in Monolayer L-Cell Cultures

*Experimental cultures treated with 0.5 ml of partially purified "IF"
*Control cultures treated with 0.5 ml of control fluids
Challenge inoculum approximately 100 pfu/culture

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	77	99
2	83	84
3	98	89
4	90	90
	Mean: 87	Mean: 88

*For details of preparations see section on Materials and Methods.

TABLE IX. Effect of * Extracellular and ** Intracellular Mengovirus "Interferon" on the Growth of Homologous Virus in L-Cell Monolayer Cultures

Experimental cultures treated with 0.5 ml of extracellular "IF".
Experimental cultures treated with 0.5 ml of intracellular "IF".
Control cultures treated with 0.5 ml of extracellular control fluids.
Control cultures treated with 0.5 ml of intracellular control fluids.
Challenge inoculum - 100 pfu/culture.

Culture No.	Plaque Count in Cultures Treated With Extra-Cellular IF. Plaque/Culture	Plaque Count in Cultures Treated With Extracellular Control Fluid Plaques/Culture	Plaque Count in Cultures Treated With Intra-Cellular "IF". Plaques/Culture	Plaque Count in Cultures Treated With Intracellular Control Fluid Plaques/Culture
1	80	70	70	66
2	77	100	60	90
3	80	40	70	76
4	50	80	80	95
	Mean: 72	Mean: 72	Mean: 70	Mean: 72

* For details of preparation see section on Materials and Methods.

took 56 hours for the inactivation of 1.6×10^5 pfu/ml of Mengovirus. Fig. 4 shows the inactivation curve of Mengovirus.

Then the experiment was done as described in Materials and Methods to find out whether heat-inactivated Mengovirus interferes with the growth of Mengovirus or not. The results are summarized in Table X. There was no interference produced between the heat-inactivated Mengovirus and the growth of Mengovirus in the cultures pretreated with heat-inactivated virus.

I. Rate of Inactivation of Mengovirus with UV Light

Before doing the experiments of the interference of UV-inactivated Mengovirus with Mengovirus, it was necessary to establish the rate of inactivation of Mengovirus with UV light. It was determined as 60 seconds at pH 7.2 for 5.0 ml in a glass culture dish of virus containing approximately 1.6×10^4 virus particles/ml. The UV-inactivation curve of Mengovirus is shown in Fig. 5.

Interference experiments with the growth of Mengovirus was also done by pretreating the cell cultures with the inactivated Mengovirus. Results are shown in Table XI. The UV-inactivated Mengovirus did not show any interference with the growth of Mengovirus in L-cells. The control cultures and UV-inactivated virus treated cultures showed almost the same number of plaques per cultures.

J. Interference with the Mengovirus by Live Newcastle Disease Virus

L-cell monolayers were infected with approximately 128 HA of NDV and then after incubating for 6 hours the cultures were challenged with approximately 100 pfu of Mengovirus per culture. The results are summarized in Table XII. After the development of the plaques, it was

INACTIVATION OF MENGOVIRUS AT 37° c.

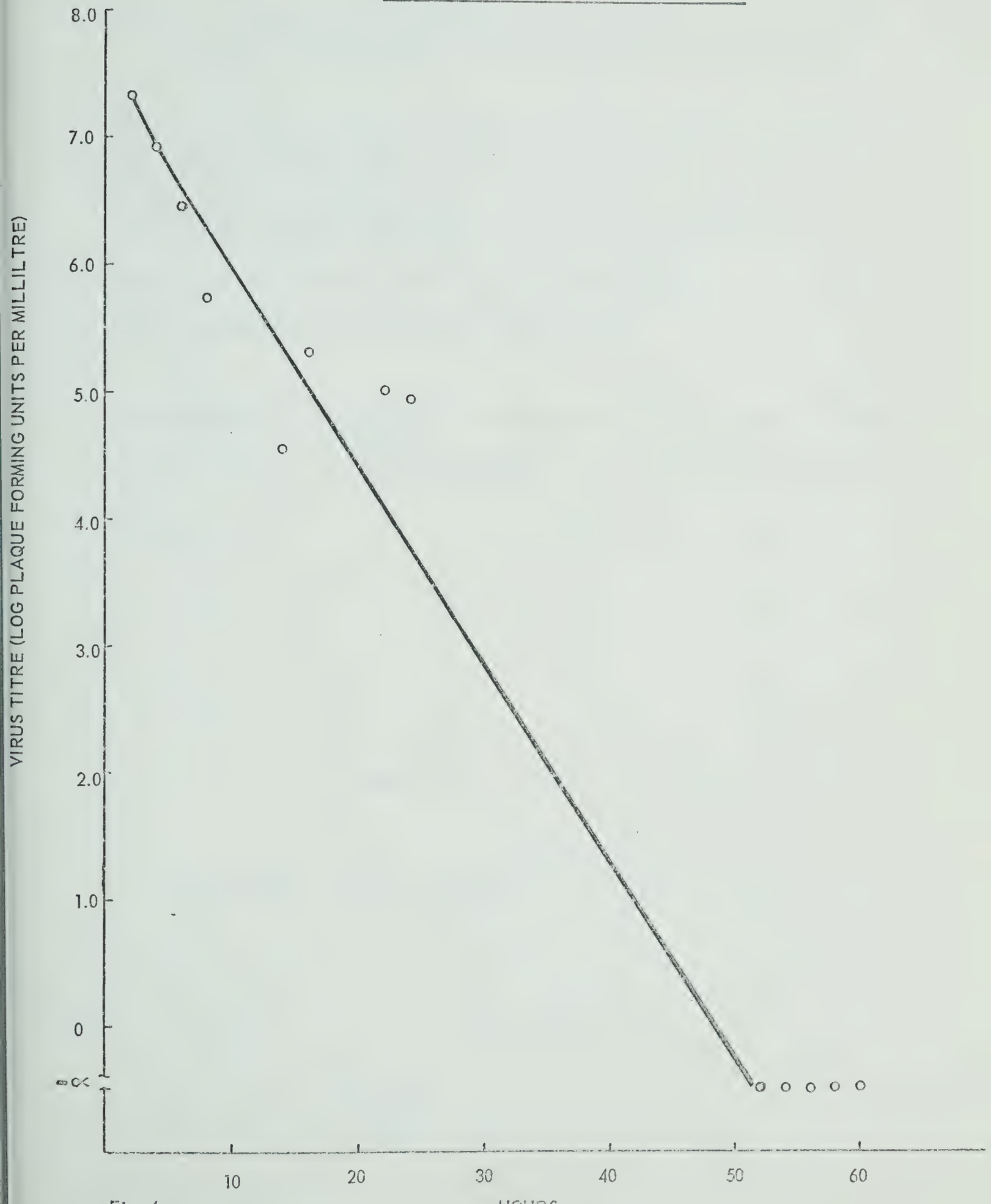


Fig. 4

TABLE X. Interference With Mengovirus After Pretreatment of L-Cell Monolayer Cultures With Heat-Inactivated Homologous Virus

Cell count - 1×10^7 cells/culture.

Experimental culture treated with 5×10^7 heat-inactivated virus particles/0.4 ml/culture.

Control culture treated with 0.4 ml of control fluids.

Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	70	46
2	91	81
3	57	57
4	46	100
	Mean: 66	Mean: 71

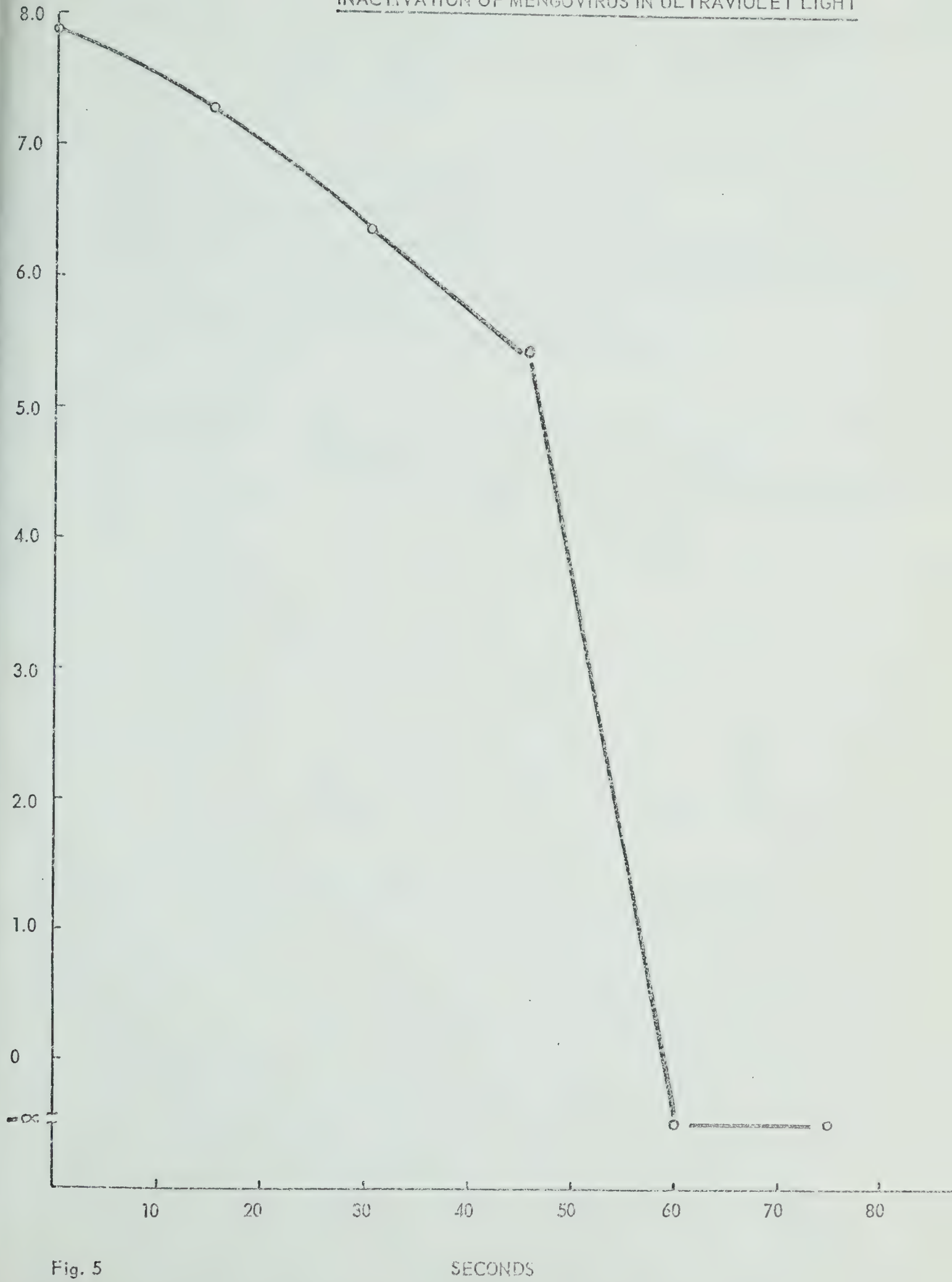

INACTIVATION OF MENGOVIRUS IN ULTRAVIOLET LIGHT
VIRUS TITRE (LOG PLAQUE FORMING UNITS PER MILLILITRE)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-∞
10
20
30
40
50
60
70
80
SECONDS

Fig. 5

TABLE XI. Replication of Mengovirus After Pretreatment of L-Cell Monolayer Cultures With Ultraviolet-Inactivated Homologous Virus

Cell count approximately 1×10^7 cells/culture.

Experimental culture treated with 5×10^7 of UV-inactivated virus particles/0.4 ml/culture. Incubated 6 hours before challenge.

Control culture treated with 0.4 ml of control fluids.

Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	60	46
2	56	41
3	70	57
4	50	100
	Mean: 59	Mean: 61

TABLE XII. Interference With Mengovirus by Live Newcastle Disease Virus in L-Cell Monolayer Cultures

Cell count approximately 1 x 10^7 cells/culture.
Experimental culture infected with 128 HA units/0.5 ml/culture.
Control culture treated with 0.5 ml of EBSS.
Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	None	63
2	None	70
3	None	75
4	None	80
	Mean: None	Mean: 72

found that the control cultures showed plaques at the average of 72 plaques per culture, whereas the cell cultures first infected with live NDV and then challenged with Mengovirus showed no plaques at all, giving evidence that NDV completely inhibited the growth of Mengovirus when the cell cultures were pretreated with live NDV.

K. Studies of the Unattached Mengovirus on Monolayer L-Cell Cultures Infected With Newcastle Disease Virus

To find out whether this interference takes place at the site of attachment of virus to the cell or inside the cell, an experiment was done to find out how much virus attached to the cells treated with live NDV. It was determined by calculating the mean unattached virus per culture both in control cultures and live NDV infected cultures. It was found that on the average, 16 pfu/culture out of 100 virus pfu/culture did not attach to the cells. The figure in the live NDV-treated cell culture was 24 unattached pfu/culture. Results are summarized in Table XIII.

L. Effect of Newcastle Disease Virus Interferon on the Growth of Mengo-Virus in L-Cell Monolayer Cultures After 6 Hours of Incubation

The detailed experiment has been described in Materials and Methods. The results are summarized in Table XIV. The results show there is reduction in the number of plaques in the IF-treated cultures as compared with the control cultures. The IF-treated cultures show a mean of 30 plaques/culture. The control cultures show a mean of 90 plaques/culture. There is approximately 70% reduction in the plaques in the IF-treated cell cultures.

TABLE XIII. Studies of the Unattached Mengovirus in *Newcastle Disease Virus Infected Monolayer L-Cell Cultures.

Inoculum - 0.2 ml/culture from 3.0 ml saved washings.

Unattached Mengovirus From NDV Infected Cultures Plaques/Culture	Unattached Mengovirus From Control Cultures Plaques/Culture
2	1
3	3
1	None
None	3
None	1
None	None
6	2
None	None

*For details of experiment see section in Materials and Methods.

TABLE XIV. Effects of Newcastle Disease Virus Interferon on the growth of Mengovirus in L-Cell Monolayer Cultures After 6 Hours of Incubation*

Experimental culture treated with 0.5 ml IF/culture.
Control cultures treated with 0.5 ml of control culture fluids.
Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	30	85
2	28	89
3	31	90
4	33	99
	Mean: 30	Mean: 90

*Cultures were treated with IF, incubated for 2 hours; cultures were then washed and incubated for another 6 hours.

M. Effect of Newcastle Disease Virus Interferon on the Growth of Mengovirus in L-Cell Monolayer Cultures After 24 Hours of Incubation.

The results of this experiment are summarized in Table XV. In IF-treated cultures an average of 23 plaques were found per culture and in control cultures a mean of 94 plaques were found per culture. There is about 77% reduction in the number of plaques.

N. Effect of Varying the Dose of Newcastle Disease Virus Interferon on the Growth of Mengovirus in L-Cell Monolayer Cultures

Results are summarized in Table XVI. Varying the dose from 1.0 to 0.5 ml does not have any effect on the reduction of plaques in L-cell cultures.

O. Effect of Mengovirus "IF" and of Partially Purified "IF" on the Growth of Vaccinia Virus in L-Cells

L-cell cultures were treated with Mengovirus IF, partially purified IF and incubated for 6 hours at 37°C after incubating at 37°C for 1 hour for the absorption of IF to L-cells. The cell cultures were then challenged with approximately 100 pfu of vaccinia virus. The experiment was described in Materials and Methods. The results are summarized in Tables XVII and XVIII. The results showed that there was no effect of either Mengovirus "IF" or partially purified "IF" on the growth of Vaccinia virus in L-cells.

P. Effect of Rabbit Anti-Mengovirus Serum and Immune γ-Globulin on Mengovirus in L-Cells

As described in Materials and Methods, RAMS was prepared from immunized rabbits and γ-globulin was prepared from RAMS by a simple chromatographic method.

TABLE XV. Effect of Newcastle Disease Virus Interferon on the Growing Mengovirus in L-Cell Monolayer Cultures After 24 Hours Incubation*

Experimental culture treated with 0.5 ml of IF/culture.
Control culture treated with 0.5 ml of control fluids/culture.
Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	28	80
2	26	100
3	18	98
4	22	100
	Mean: 23	Mean: 94

*Cultures were treated with 0.5 ml of IF, incubated for two hours, cultures were washed and incubated for another 24 hours.

TABLE XVI. Effect of Varying the Dose of Newcastle Disease Virus Interferon on the Growth of Mengovirus in Monolayer L-Cell Cultures.

Experimental culture treated with 1.0 ml of IF/culture.
Experimental culture treated with 0.5 ml of IF/culture.
Control culture treated with 1.0 ml of control culture fluids.
Control culture treated with 0.5 ml of control culture fluids.

Challenge inoculum approximately 100 pfu/culture.

Culture No.	Experimental Cultures Treated with 1.0 ml IF.	Control Cultures Treated with 1.0 ml of Control Culture Fluids.	Experimental Cultures Treated with 0.5 ml of IF.	Control Cultures Treated with 0.5 ml of Control Culture Fluids.
1	23	99	37	80
2	33	100	32	98
3	37	100	32	99
4	36	75	36	90
	34	93	34	92

TABLE XVII. Effect of Mengovirus "Interferon" on the Growth of Vaccinia Virus in Monolayer L-Cell Cultures

Experimental cultures treated with 0.5 ml of "IF"/culture.
Control cultures treated with 0.5 ml of control fluids/culture.
Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	91	80
2	69	82
3	70	92
4	84	90
	Mean: 79	Mean: 86

TABLE XVIII. Effect of partially purified Mengovirus "Interferon" on the Growth of Vaccinia Virus in L-Cell Monolayer Cultures.

Experimental cultures treated with 0.5 ml of "IF"/culture.

Control cultures treated with 0.5 ml of control fluids/culture.

Challenge inoculum approximately 100 pfu/culture.

Culture Number	Plaque Count in Experimental Cultures Plaques/Culture	Plaque Count in Control Cultures Plaques/Culture
1	70	75
2	65	50
3	49	54
4	53	69
	Mean: 59	Mean: 62

Before infecting the cell cultures with Mengovirus, the virus and the RAMS mixture was kept at 37°C for 1 hour and the virus plus immune γ-globulin mixture was also kept at 37°C for 1 hour so as to facilitate the neutralization of the virus.

The cultures were then infected with these mixtures and after 72 hours plaque assays were done. The results of this experiment are summarized in Table XIX. The results show that the control cultures showed approximately 26 plaques per culture. The other cultures, which were infected with either a mixture of virus and RAMS or a mixture of virus and immune γ-globulin showed no plaques at all.

Another experiment was done the same way with RS and normal γ-globulin. The results are summarized in Table XX. All the cultures showed approximately the same number of plaques.

Q. Effect of Rabbit Anti-Mengovirus Serum, Immune γ-Globulin, Rabbit Normal Serum and Normal γ-Globulin on (IRNA) in L-Cells.

This experiment was also done as for the Mengovirus and all the detailed experimental method has been described in Methods. This experiment was done to find out whether there was any RNase activity in RAMS, immune γ-globulin, RS and RS γ-globulin. The results are summarized in Tablex XXI and XXII. The results show that both types of serums and both types of γ-globulins neutralized IRNA.

R. Specific Effect of Serum Enzymes on Tritium Labelled IRNA.

Since the foregoing results showed that IRNA could be neutralized by serum or γ-globulin which did not contain specific antibodies an experiment was done to determine whether or not the effect was due to serum

TABLE XIX. Neutralization of Mengovirus by Rabbit Anti-Mengovirus Serum and Immune γ-Globulin.

*Experimental cultures treated with a mixture of 0.2 ml of RAMS and 0.2 ml of virus/culture.

**Experimental cultures treated with a mixture of 0.2 ml of immune γ-globulin and 0.2 ml of virus.

***Control cultures infected with approximately 30 pfu/culture.

Culture No.	Experimental Cultures Treated With a Mixture of RAMS and Virus. Plaques/Culture	Experimental Cultures Treated With a Mixture of Immune γ-Globulin and Virus. Plaques/Culture	Control Cultures Plaques/Culture
1	None	None	27
2	None	None	30
3	None	None	24
4	None	None	24
Mean:	None	Mean: None	Mean: 26

***For details see section on Materials and Methods.

TABLE XX. Effect of Rabbit Serum and Rabbit γ-Globulin on Mengovirus in Monolayer L-Cell Cultures.

*Experimental cultures treated with a mixture of 0.2 ml RS and 0.2 ml RS and 0.2 ml virus/culture.

**Experimental cultures treated with a mixture of 0.2 ml of γ-globulin and 0.2 ml/culture.

***Control cultures infected with approximately 30 pfu/culture.

Culture No.	Experimental Cultures Treated With a Mixture of RS and Virus. Plaques/Culture	Experimental Cultures Treated With a Mixture of γ-Globulin and Virus. Plaques/Culture	Control Cultures Plaques/Culture
1	20	31	30
2	18	19	35
3	22	17	25
4	23	24	25
	Mean: 21	Mean: 23	Mean: 29

⁂For details see section on Materials and Methods.

TABLE XXI. Effect of Rabbit Anti-Mengo Serum and Immune γ-Globulin on Mengovirus Infectious Ribonucleic Acid in Monolayer L-Cell Cultures.

*Experimental culture treated with a mixture of 0.2 ml of RAMS and 0.2 ml of IRNA culture.

**Experimental culture treated with a mixture of 0.2 ml of immune γ-globulin and 0.2 ml of IRNA/culture.

***Control cultures infected with approximately 30 pfu/culture.

Culture No.	Experimental Cultures Treated With a Mixture of RAMS and IRNA Plaques/Culture	Experimental Cultures Treated With a Mixture of Immune γ-Globulin and IRNA. Plaques/Culture	Control Cultures Plaques/Culture
1	None	None	30
2	None	None	30
3	None	None	31
4	None	None	29
	Mean: None	Mean: None	Mean: 30

⁂For details of experiment see section on Materials and Methods.

TABLE XXII. Effect of Rabbit Serum and Rabbit γ-Globulin on Mengovirus Infectious Ribonucleic Acid in Monolayer L-Cell Cultures

*Experimental culture treated with a mixture of 0.2 ml of RS and 0.2 ml of IRNA/culture.

**Experimental culture treated with a mixture of 0.2 ml of γ-globulin and 0.2 ml of IRNA/culture.

***Control cultures infected with approximately 30 pfu/culture.

Culture No.	Experimental Culture Treated With a Mixture of RS and IRNA. Plaques/Culture	Experimental Cultures Treated With a Mixture of γ-globulin and IRNA. Plaques/Culture	Control Cultures Plaques/Culture
1	None	None	30
2	None	None	30
3	None	None	28
4	None	None	25
Mean:	None	Mean: None	Mean: 28

⁂For details of experiment see section on Materials and Methods.

enzymes. Purified tritium labelled Mengovirus IRNA (IRNA*) was incubated with either RAMS or immune γ-globulin, or RS or normal globulin for 15 minutes at 37°C. The products of incubation were then subjected to centrifugation to equilibrium in sucrose density gradient, the degradation of IRNA* was followed by distribution of radioactivity. The results are summarized in Fig. 6. In the controls, the radioactive label was found to be concentrated in the centre of the tube. This is the position expected for undegraded ribonucleic acid in this system. However, in the experimental mixtures, most of the radioactive material was found at the top of the tube. This distribution of the label indicated that the labelled ribonucleic acid has been broken down into small fragments. There is one unexpected finding. A concentration of labelled ribonucleic acid was found to be present at the bottom of the tube in the case of ribonucleic acid incubated with immune γ-globulin.

S. Demonstration of Ribonuclease Activity by the Diffusion Plate Method

The nuclease activity in RAMS and RS was demonstrated by diffusion plate method using commercial RNA as substrate. The immune γ-globulin and normal γ-globulin also retained their nuclease activities as demonstrated by the diffusion plate method. Aliquots (0.2 ml) of RAMS and immune γ-globulin were added to antibiotic assay cups embedded in diffusion plate assay agar and the plate incubated and developed as described in Materials and Methods. The results are shown in Fig. 7 and Fig. 8. Nuclease was present in both RAMS and immune γ-globulin which was revealed as a clear, sharply defined zone which surrounded the assay cup in the developed plate. The radius of the zone for the activity of the enzyme present in RAMS was

DISTRIBUTION OF RADIOACTIVITY IN DENSITY GRADIENTS FOLLOWING INCUBATION OF IRNA* WITH:

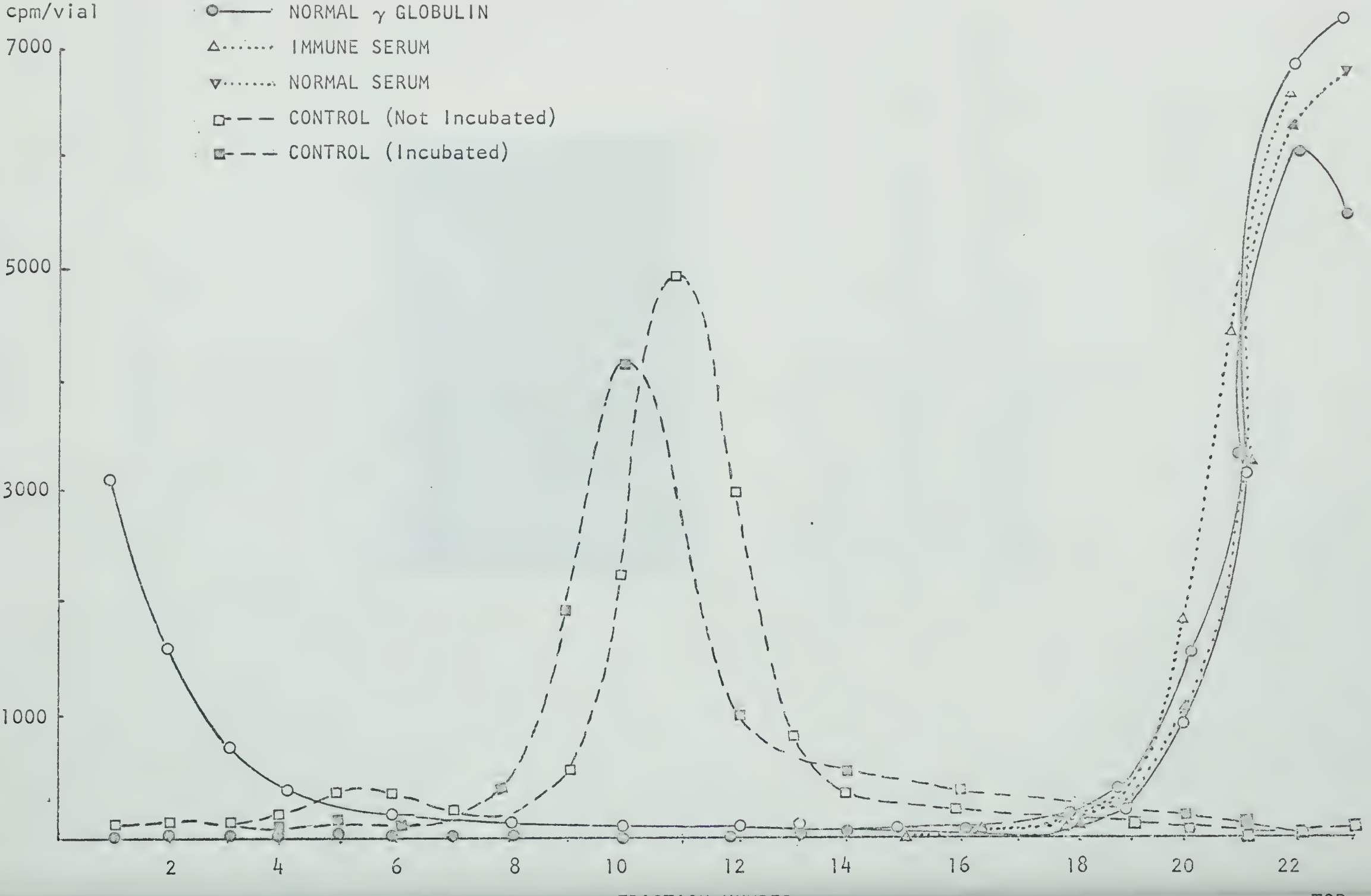
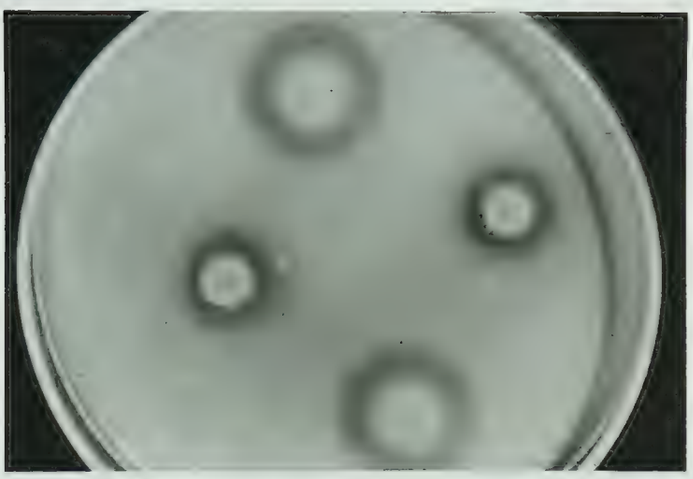


Figure 7

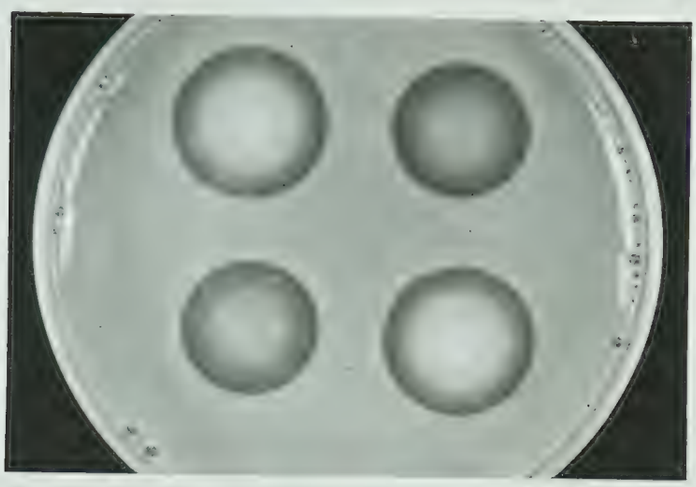


Demonstration of Ribonuclease Activity by Diffusion Plate Method.

1. Clear sharply defined zones of about 5-6 mm in radius formed as a result of enzyme present in rabbit anti-Mengo virus serum.

2. Clear sharply defined zones of about 3-4 mm in radius formed as a result of enzyme present in immune γ-globulin.

Figure 8



Demonstration of Ribonuclease Activity by Diffusion Plate Method.

1. Clear sharply defined zones of about 6-7 mm in radius formed as a result of enzyme present in rabbit normal serum.

2. Clear sharply defined zones of about 4-5 mm in radius formed as a result of enzyme present in normal γ-globulin.

approximately 5-6 mm, for the enzyme activity present in immune γ-globulin it was 3-4 mm. The same type of experiment was done to reveal the nuclease activity present in RS and normal γ-globulin. Both the RS and normal γ-globulin showed nuclease activity, the radius of the zones showing the enzyme activity present in RS was 6-7 mm and for the zone showing the activity of enzyme present in normal γ-globulin it was 4-5 mm.

Another diffusion plate method was done by placing cellulose acetate strips on the plates after subjecting them to electrophoresis and drying them. The other half of the cut strip was stained in Ponceau to reveal proteins and placed along for comparison. The method was described in Materials and Methods. The plates were incubated and developed. The plate on which the RAMS strip was placed showed the clear area from the point of origin of bands and to the end of the bands. The length of the clear area as measured was approximately 3.5 cm. The length of the bands was also approximately 3.5 cm. About 2.3 cm of the total clear area was very well marked but the rest of it was faintly clear. In the case of RS, the clear area was seen along with the length of bands but activity is greater at the site of albumin. The length of clear area was about 5 cm. The same experiment was done with immune γ-globulin, and normal γ-globulin, but with very little activity.

DISCUSSION

Discussion

A. Preliminary Studies

Plaque assay of the virus in L-cell cultures revealed plaques of about 2-3 mm in diameter, almost round, and essentially similar to the plaques formed by Mengovirus, strain M, as described by Ellem and Colter (1961). Under the standard conditions of attachment used in all experiments reported in this thesis, maximum virus attachment (about 85%) occurred within one hour after infecting the cells. A similar rate of attachment has been reported by Brownstein and Graham (1961) who showed that 70% of Mengo 37A attached to L-cell monolayers within 45 minutes.

Virus yield was not studied in detail at times less than nine hours following infection mainly because these time intervals were not of interest in the experiments which followed.

B. Factors Which Might Control the Propagation of Mengovirus in L-Cells

(a) An attempt was made to produce "M"-Mengovirus interferon in L-cells by infecting the cells with live Mengovirus and then following a generally accepted method for the preparation of IF.

When L-cell monolayer cultures were treated with "IF" prior to challenge with Mengovirus no interference with plaque-forming ability was shown. Even when extended time intervals were given for the incubation of L-cell cultures and "IF" (from 6, 12, 18, 24 hours) prior to the challenge of Mengovirus no interference was caused. The yield of Mengovirus was also measured at 9 hours, 18 hours, and 27 hours after treating the cells with "IF". "Interferon" had no effect on the yield of Mengovirus at various time intervals.

Studies were also done on the attachment of Mengovirus to "IF"-pre-treated cells. The same number of infective particles attached in control cultures as in "IF"-treated cultures. No interference was demonstrated at the site of attachment. This result is consistent with those reported above.

The results of all these studies indicated that perhaps live Mengovirus is a very poor inducer of IF in L-cell cultures. However, there might have been some impurities in the preparation which inhibited the action of "IF". To shed some light on this latter possibility, "IF" was partially purified and the effect of this preparation on the growth of Mengovirus was tested. Even partially purified "IF" did not interfere with the growth of Mengovirus in L-cell cultures.

Finally, intracellular "IF" was prepared as described in Materials and Methods. This preparation, too, was found to be inactive.

These consistent negative results suggested that Mengovirus is a very poor inducer of IF in L-cell cultures. Perhaps a very small amount of IF was produced which could not be detected in the system used. This possibility could be explained on the basis of fundamental work done by various investigators. It has been reported that DNA-controlled RNA synthesis is required for the formation of IF. Burke and Morrison (1966) have shown that it was not really synthesis of DNA which was required for the formation but it was RNA. Wagner (1964) working with NDV infection in mouse tumour cells have shown that the time for the formation of m-RNA was about 6 hours. Levy and associates (1965) found that the time for the formation of m-RNA for IF formation in chick embryo cultures infected Chikungunga virus was 1-1/2 - 2 hours after infection.

Baltimore, Franklin and Callender (1963) have shown that Mengovirus infection of L-cells induces a rapid decline in the rate of synthesis of both cellular RNA and protein. McCormick and Penman (1967) have also showed inhibition of cellular RNA in L-cell cultures by Mengovirus. They have shown distinctly that cellular m-RNA was also suppressed.

The rapid suppression of cellular RNA synthesis in L-cells by Mengovirus could cause very poor production of interferon, as synthesis of both m-RNA and protein is required for the production of IF. From the results presented here and from the related data, it could be concluded that live Mengovirus is a very poor inducer of IF in L-cell cultures.

It was thought that perhaps inactivated Mengovirus would be a better source of interference than the live virus. It is generally accepted that inactivated viruses for a certain system can be a better source than live viruses for the production of interference. For example, Burke and Isaacs (1958) showed that heat-inactivated influenza virus and UV-inactivated influenza viruses are better sources of IF production as compared with live influenza virus in CAM. Also, they found that UV-inactivated influenza virus was the best stimulus for the production of IF in CAM. Therefore, preparations of both heat-inactivated and UV-inactivated Mengoviruses were tested for their ability to interfere with the growth of Mengovirus. In spite of the theoretical possibilities, the results reported here for the interference between the inactivated Mengo virus (by heat or UV) and infectious Mengovirus, showed no interference caused by inactivated virus in L-cell cultures.

The inability of heat-inactivated and UV-inactivated Mengovirus to produce interference could be due to the destruction of interfering

activity due to over exposure to heat or to UV irradiation. The aim of the inactivation of the virus is to destroy its infectivity and retain its interfering activity. In the studies done here, the interfering activity of the virus might have been destroyed in both cases. It is just as probable, however, that the inactivated virus particles at no time possessed the ability to stimulate interferon production in L-cells.

Mengovirus was found to be incapable, also, of causing interference with the plaque-forming ability of vaccinia virus in L-cell monolayer cultures. This suggests that the "interferon defect" is not peculiar to effects against the homologous virus.

Mengovirus proved to be sensitive to heterologous interference. Complete inhibition of Mengovirus replication, as well as suppression of cytopathic effect, followed exposure of the test cultures to live NDV. A preparation of NDV-IF was observed to be active against Mengovirus. There was approximately a 70% reduction in the number of plaques formed in test cultures. Increasing the time of preincubation or doubling the dose (not the concentration) of interferon did not have any apparent effect in further plaque reduction. These results are quite compatible with those reported by Guantt and Lockart (1966). These investigators studied the inhibition of Mengovirus in L-cell sublines (Lts) by IF produced by live NDV in Lts cells. They demonstrated that the yield of virus at 24 hours was decreased by 85% and there was a reduction in the size as well as the number of plaques. The reduction in plaque size was not observed in the work reported here. This difference might be due to the fact that Guantt and Lockart used a concentrated preparation of IF.

It had been known previously that Mengovirus was susceptible to interferon produced by West Nile virus in mice (Vainio, Gwatkin and Koprowski, 1961) and to interferon stimulated by Chikungunga virus in L-cells (Levy, 1964). However, the most recent study pertaining to the mechanism of action of NDV interferon is that of Gordon, Chenault, Stevenson and Acton (1966). They reported that IF produced by UV-inactivated NDV in L-cell cultures inhibited polymerization of all classes of RNA that could be presumed to be coded by the Mengovirus genome.

(b) Neutralization of Mengovirus and depolymerization of infective RNA.

Anti-serum (RAMS) prepared from blood taken from immunized rabbits, was shown to neutralize Mengovirus. Purified γ-globulin (immune γ-globulin) prepared from RAMS was also found to neutralize Mengovirus. Mengovirus was not neutralized by normal rabbit serum (RS) or by γ-globulin (normal γ-globulin) prepared from RS.

However, it was found that IRNA was rendered biologically inactive (non-infective) following exposure to RS, normal γ-globulin, RAMS or immune γ-globulin. The reason for this phenomenon was found to be the fact that all of these preparations contained RNase activity. This activity was demonstrated using a diffusion plate assay method with commercial RNA as substrate.

A highly specific RNase effect was demonstrated using Tritium-labelled purified IRNA (IRNA*) as substrate, the products of reaction being recovered in a sucrose gradient (Fig. 6). In all experimental studies, radioactivity was found to be concentrated in the top of the density

gradient whereas the unreacted controls showed the distribution characteristics of undegraded RNA. The size of the RNA fragments cannot be estimated from these results. However, Blackmore, Donovan and Pochynok (unpublished results) have shown that an endonuclease activity, widely distributed in bovine, foetal bovine and human sera causes the depolymerization of RNA into fragments larger than tri-nucleotides and smaller than octanucleotides. Although rabbit serum has not been examined in detail, preliminary studies, not reported in this thesis, indicate that similar distribution of nuclease activity is present in rabbit serum.

Attention is drawn to what seems to be an exception to the consistent experimental results described above. When immune γ-globulin was allowed to react with IRNA* prior to centrifugation to equilibrium a significant amount of label was detected at the bottom of the density gradient. This apparent anomaly will be dealt with later in the discussion.

Historically, Gierer and Schramm (1956) showed that when tobacco-mosaic virus (TMV) or its IRNA were treated with anti-serum prepared in rabbits only the virus was inactivated. It is possible that TMV-IRNA is not susceptible to the action of rabbit serum nuclease. This would be an interesting experiment to repeat. At a later date, Colter, Bird and Brown (1957) showed that Mengo IRNA was inactivated by either normal monkey serum or monkey anti-Mengo serum. More recently, Brown, Ellem and Colter (1960) reported the effect of 3 different proteins on IRNA. Bovine γ-globulin was reported to have a greater inactivating effect than bovine albumin or α-lactalbumin.

The results reported in this thesis find best agreement with work

reported by Alexander, Koch; Mountain and VanDamme (1958). They found that Polio virus Type I and II was completely inactivated only by specific anti-serum whereas normal monkey could inactivate polio IRNA. Normal rabbit serum possessed this same activity. However, globulin prepared from normal serum and anti-polio serum did not inactivate IRNA. Since the globulin was prepared by precipitation with ammonium sulphate it is possible that nuclease activity was not retained. It is quite definite, however, that in the present work the purified γ-globulin retained RNase activity which was responsible for the results obtained.

Returning now to a consideration of the portion of IRNA* which apparently had interacted with immune γ-globulin (Figure 6). It is interesting to note that Panijel and Cayeux (1966) have found that when an animal is subjected to prolonged immunization with ribosomes of whatever origin, it becomes progressively endowed with anti-nucleic acid globulin (NGI) which precipitates various types of ribonucleic acids and all synthetic polynucleotides. On the other hand, these sera and sera of animals not immunized against ribosomes contain globulins (NGII) which precipitate polyinosinic acid and polyuridylic acid. The Mengovirus preparation used to immunize the rabbits which provided the anti-serum from which the immune γ-globulin was prepared could be expected to contain polyribosomes. It is possible, therefore, that NGI was present in the immune γ-globulins and that the activity present in the bottom of the density gradient represents an IRNA*-NGI complex. This possibility remains to be investigated.

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